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A TEXTBOOK
OF
PLANT PHYSIOLOGY

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(*From a portrait by Streblow*)

Dr. N. A. MAXIMOV

A TEXTBOOK
OF
PLANT PHYSIOLOGY

BY

N. A. MAXIMOV

Professor of the Pedagogical Institute of Leningrad and Director of Plant Physiology of the Bureau of Applied Botany, U. S. S. R.

TRANSLATED FROM THE RUSSIAN

EDITED BY

A. E. MURNEEK AND R. B. HARVEY

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PREFACE TO THE ENGLISH TRANSLATION

When Dr. R. B. Harvey was my guest in Leningrad in 1927 I showed him my "Textbook of Plant Physiology" which had just been printed. He was interested in the book and asked me whether I did not expect to prepare an English translation of it. I answered that I had not been thinking about this, but when another of my guests from America, Dr. William Seifriz, also advised me to issue this textbook in the United States, I determined to follow this advice.

The translation of the book was done at my request by the following: Miss Alma Trost (Chaps. I, IV–VII, IX, and X), Mrs. Isabella Gerbatch (Chaps. II, XI, and XII), Mrs. Irene Krassovsky (Chap. III), all of Leningrad, and Dr. Michael Shapovalov (Chap. VIII), of Riverside, Calif. To my great satisfaction my friend, Dr. A. E. Murneek, who is well acquainted with the Russian language, consented to edit the translation. His task has not been an easy one, for though the translators had made an exact copy of the Russian into English, nevertheless the editor had to revise the translation thoroughly in order to coordinate the expressions of the different translators and to minimize as much as possible the influence of the Russian on the English style. Dr. Murneek has carried out this work with love and perseverance. Almost the whole text had to be worked over and partly rewritten and, as far as I can judge, the book has quite lost the character of a translation.

When the English manuscript was almost ready for publication the first Russian edition was out of print and it was necessary to prepare a second. This edition contains several alterations and changes which are included in the English text. Dr. R. B. Harvey has edited these supplements and has critically reedited the whole text. Thus the English translation was edited by two authoritative American workers in plant physiology. This gives me the certainty that, thanks to their efforts, the book, though written in Russian, has been successfully transformed into an English text.

I express my sincere appreciation to all the persons mentioned

above for their help in this difficult undertaking—of making my book accessible to students of the English-speaking countries. I hope that it will meet with the same kind reception there that it has in Russia.

N. A. Maximov.

Leningrad
May, 1930.

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EDITORS' NOTE

For several years there has been a need for an English textbook of plant physiology which presents the fundamentals of the physiology of plants and which gives examples of the phases of this subject as applied to the various branches of agriculture and forestry. Dr. N. A. Maximov's textbook seems to fulfill this need in an excellent manner. It presents thoroughly and clearly all the important features of plant physiology that a beginning student should know.

The book has certain pedagogical merits. It is arranged into four major parts with conveniently short chapters, thus facilitating its use for class work. The attention of the student is not detracted by too numerous references to literature nor by too profuse explanatory footnotes, yet sufficient reference is made to names prominent in the historical development of each subject. Illustrations and data are often taken from the application of physiology to practical problems. In many cases American literature has been used as the source of illustrative material. The data presented from Russian literature offer an introduction to many valuable plant physiological contributions which formerly have not been available to students on account of the difficulty of reading the Russian language. The text brings up to date the results of physiological research both in Russia and in the United States.

The personal interest of the author in temperature, light and water relations in plants, and in photosynthesis has been reflected in a most desirable manner in this textbook. The position of Dr. Maximov as director of plant physiological work in the Institute of Applied Botany of the Soviet government has brought him into contact with many practical aspects of plant physiology. Russia is a region which offers an unusual range of ecological conditions. The physiological effects of these extremes of environment have been the field of Maximov's experimentation. His research work on cold and drouth resistance in plants has made him an outstanding scientist. It is especially valuable to have so thorough a discussion on frost injury and drouth effects in plants by one

who has had much to do with the development of research in these fields.

Translations from Russian into English are difficult on account of the great differences in habit of thought and action of the two peoples. The style, arrangement, and character of the original Russian have been preserved in this translation in so far as possible for clearness. The style of the English has been modified therein. All corrections have been inserted from the second Russian edition, which is soon to be published. Dr. Maximov and Mme. T. Krassnoselsky-Maximov have read the edited copy.

University of Missouri. A. E. Murneek.
University of Minnesota. R. B. Harvey.

September, 1930.

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A TEXTBOOK
OF
PLANT PHYSIOLOGY

INTRODUCTION

Plant physiology is a science that deals with the vital phenomena that occur in plants. Its objects are, first, to subdivide into separate functions the complex activities that constitute the life of a plant; second, to establish the rôle of each of these in the general behavior of the organism; and, finally, to analyze, as thoroughly as possible, every process into its simpler constituent parts, *i.e.*, into reactions that may be observed even in the inorganic world.

Plant physiology is an inseparable part of botany. It is closely connected with the study of morphology and anatomy of plants, since without a sufficient knowledge of plant structure an effective study of physiology is hardly possible. Recently plant physiology has been forming connections not only with plant ecology and plant geography but also with taxonomy. Plant physiology is passing gradually from the study of activities common to all plants to a consideration of the physiological distinctions that exist between various groups or even species.

Though being a part of the botanical sciences, plant physiology is also closely linked with animal physiology. Many essential organic functions, such as respiration, nutrition, and irritability, are exhibited both by plants and by animals. Hence, the numerous attempts to create a science of general physiology that should include the study of vital activities manifested by all organisms. Such an approach to the investigation of living beings, however, would leave out of consideration the specific nature of plants. An independent study of the problems that refer particularly to the physiology of plants, therefore, is necessary.

Plant physiology differs from the other more general biological sciences, in which description plays an important part, in that it is

based particularly on the physicochemical sciences. When a detailed investigation in physiology is undertaken, the field of biology is usually left behind and the realm of physics and chemistry is entered. In the course of such a study difficulties of two kinds are encountered. In the first place, life activities may be characterized by such complexities that it is very difficult to subdivide them completely into their physiological components. And, considering the present state of our knowledge, it is often impossible to subject them entirely to the laws of physics and of chemistry. This is particularly true of many problems pertaining to the physiology of growth and reproduction. Secondly, some of the physicochemical sciences that are most closely connected with physiology, as, for instance, physical and especially colloidal chemistry, are as yet insufficiently developed.

The first type of difficulties has prompted certain investigators to take a pessimistic attitude toward the possibility of ever carrying this analysis to an ultimate end. It has led them to believe that in living organisms there exists a certain "something" which cannot be subjected to a physicochemical analysis. This "something" was once defined as a "vital force," and this explanation has become known by the name "vitalism." The theory of vitalism is opposed to the mechanistic conception of life, which regards the organism as a physicochemical mechanism of very complex character, to be sure, but nevertheless fundamentally not differing from non-living things.

The adherents of the mechanistic point of view, to which belong the majority of the present-day physiologists, are convinced that eventually it will be possible to analyze the most complex vital phenomena into their physicochemical components. The differences of opinion that exist between the vitalists and the mechanists, of course, finally will be ironed out. But this may happen only in the distant future when all the organic functions become completely clear. One must emphasize the fact that the history of plant physiology seems to be a history of victories gained for the mechanistic theory. Moreover, the mechanistic principles are far more helpful as working hypotheses to the investigators, since they call for a thorough analysis of all phenomena.

Our present incomplete knowledge of the adjoining fields of physics and chemistry does not necessarily prevent physiologists from carrying on their work. There are many instances on record where physiologists, being prompted by the requirements of

their own science, have studied such phases of physics and chemistry as were most important for the solution of a particular problem. Pfeffer's famous osmotic investigation is a well-known illustration of such a case. Starting primarily with studies of the mechanism which regulates the motion of the leaves of *Mimosa*, he finally formulated some of the most fundamental conceptions for the theory of solutions. Similar profitable excursions into the adjoining fields of science, however, are but rare exceptions. One must acknowledge, nevertheless, that progress in the science of plant physiology, as a rule, is closely connected with the development of the physicochemical sciences. Every important discovery in physics and chemistry seems to bring new enthusiasm and activity into physiological investigations by opening new possibilities. A good case was the discovery of oxygen at the beginning of the science of plant physiology. This enabled physiologists to understand correctly for the first time the basis of plant nutrition. A similar instance can be observed at present, when the study of the concentration of H^+ ions has become of great value to physiology.

A close connection between physiology and chemistry has led to the development of an intermediate science, which has been named *physiological chemistry* or *biochemistry*. It consists primarily of the study of chemical transformations within the organism or related to it. Many physiological problems at present are being transferred to the field of biochemistry. This has led some scientists to believe that biochemistry will finally replace physiology. This, of course, is not true. By transferring to biochemistry a part of its problems of an analytical nature, physiology will only gain. This will enable plant physiologists to devote more attention than formerly to the very essential problem of establishing a connection between the separate biochemical and biophysical processes and the uniting of these into a harmonious system, called life.

Plant physiology serves as one of the most important foundations for the agricultural sciences. An advance in physiology, therefore, frequently leads to new progress in the art and science of crop production. And, on the other hand, it often happens that agricultural problems serve as stimuli for an increased study of particular phases of physiology. Plant physiology is certainly indebted to the agronomists for their contributions to the theory of plant nutrition. The names of such agricultural scientists as Boussingault, Hellriegel, and Prianishnikov rank among the most

famous in the history of plant physiology. This connection between agriculture and plant physiology provides the latter repeatedly with new vitality for its future development.

To the casual observer the life of a plant may appear more simple than that of the visibly more active animal. From the biochemical point of view, however, the transformations within a plant are far more varied and complex than those in the animal. Plants alone are able to manufacture organic compounds from such elementary substances as carbon dioxide, water, and the mineral salts. This important synthesis requires a considerable amount of energy, which plants obtain from sunlight.

Plant physiology deals, therefore, in the first place, with the study of the synthetic mechanisms, which are characteristic of plants and are the initial steps in their nutrition. This phase of the subject matter will be discussed in the first part of the book.

The synthesis of organic compounds from inorganic constituents takes place in the leaves, wherein a continual exchange of gases with the surrounding atmosphere takes place. But owing to their structure, leaves necessarily evaporate water, which seems to be indispensable for their normal activity. The absorption and release of water, therefore, are of great importance in the life of a plant. The second part of the book deals with water relations.

In the third part the detailed steps in the nutrition of plants will be discussed. It will be concerned primarily with the absorption and utilization of compounds prepared by primary synthesis and the production of new masses of living matter. This will lead to the study of the mechanism of respiration and the liberation of energy. Since the essential features of nutrition and respiration are common to both plants and animals, we shall come in close contact with problems of general physiology.

The utilization of nutritive substances results in the growth, movement, and reproduction of organisms. These again are general physiological problems, but in plants they are characterized by considerable specificity. These and related questions are discussed in the fourth and last part of the book. An advance much beyond a description of the phenomena occurring has not been made in this phase of plant physiology. Still, because of their great theoretical importance, it has been deemed necessary to treat them, even in this brief text, in considerably greater detail than is customary.

Part I

ABSORPTION OF MATTER AND ENERGY

CHAPTER I

ABSORPTION OF CARBON AND ACCUMULATION OF POTENTIAL ENERGY

1. Sources of Carbon for the Plant. Method of Artificial Cultures.—Plant life is characterized by continuous accumulation of organic compounds. These compounds are not absorbed from the environment in an elaborated condition. They are synthesized in the body of the plant itself, from simpler substances taken by the plant from its surroundings. The absorbing organs of the plant are the roots and leaves. Roots absorb from the soil, water and the substances dissolved in it, while leaves take in gaseous substances from the atmosphere.

The organic compounds constituting the body of a plant are rather complex in their composition. We may subject plants to analyses of two kinds—an organic analysis and an elementary one. In organic analysis we endeavor to single out, in a state as unaltered as possible, the chemical compounds whose complex mixture constitutes the body of the plant. These are in the first place the carbohydrates, such as cellulose, starch, and the different sugars. Cellulose forms the framework of the plant, the cell walls. The other carbohydrates serve as reserve substances. Fats and proteins are present in smaller amounts serving as constituents of protoplasm and as reserve nutrient compounds. Still less in amount are the pigments which produce the color of plants. The presence of organic acids, glucosides and alkaloids frequently impart to the plant an acrid flavor and poisonous properties. The detailed examination of these and numerous other substances, such as the tannins, resins, ethereal oils, etc., constitutes organic or biological chemistry. Essential components of the plant body also are the different inorganic salts which are partly dissolved in the cell sap, and are found in the protoplasm or in the cell wall. Most easily detected in the plant are the salts of calcium, the carbonates, and especially oxalates. These are deposited in the cells in crystalline formations, described in plant anatomy. By means of sensi-

tive microchemical reactions it is not difficult to demonstrate the K, Mg, Na, SO_4, PO_4, and other ions in the plant.

Besides an organic analysis, it is possible also to make an elementary analysis, *i.e.*, to try to ascertain which chemical elements are present and in what quantitative proportions they make up the body of a plant. For this purpose it is necessary first to dry the plant, since no less than 80 per cent of it is water, and then to burn with oxygen the resulting dry matter, according to the accepted methods of organic chemistry. In a great majority of cases, it has been found that almost half of the dry matter consists of carbon, and almost an equal amount of oxygen. Hydrogen, and nitrogen are present in considerably smaller quantities. In addition to the elements volatilized on combustion, there remains a certain amount of non-volatile compounds in the form of ash. The average composition of the dried plant tissues may be expressed as follows:

C, 45 per cent	N, 1.5 per cent
O, 42 per cent	Ash, 5 per cent
H, 6.5 per cent	

Different plants and different parts of plants vary considerably in their composition, hence the above-mentioned values must not be regarded as absolute. The composition of ash is also very complex and varied. Avoiding details, to which we shall return in Chapter III, it must be remarked here that the following metals are always found in ash: potassium, sodium, calcium, magnesium, iron, and manganese; and of non-metals, sulphur, phosphorus, silica, and chlorine.

When plants are grown under natural conditions, in a soil comparatively rich in humus, it is not possible to determine definitely the source of the principal constituents. All these elements may be found in the soil in the form of different organic and mineral compounds. Some of them, namely nitrogen, oxygen, and carbon (as carbon dioxide) are present also in the atmosphere. The long continued observations of agriculturists, that plants develop well and produce high yields only when grown on dark soil rich in humus, led to the conviction that all of the necessary substances are obtained by the plant from the soil nutrients. This conviction, however, has proven to be erroneous, as may be demonstrated best by the method of artificial cultures.

This method consists in growing plants in an artificial medium, the constituents of which are exactly determined, instead of in natural soil whose composition is very indefinite and difficult to ascertain. Pure quartz sand, small beads, or even platinum chips may serve as such a medium, but water cultures are the simplest and most convenient. In this case, the roots of the plant are not in soil but in water, in which are dissolved all of the elements that the plant is not able to obtain from the atmosphere but which are necessary for its development.

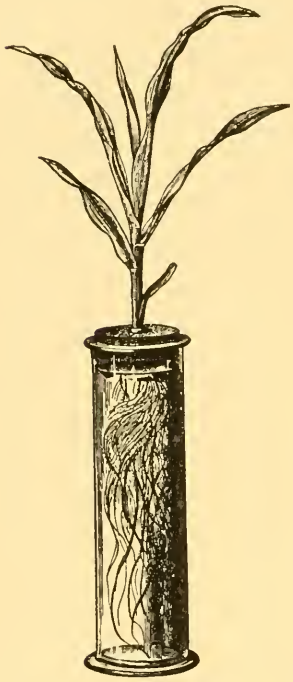


Fig. 1.—A water culture (*after Nathansohn*).

Experiments first conducted in the middle of the nineteenth century and repeated many times afterwards, have shown definitely that for the normal development of the plant only a limited number of elements must be introduced into the solution. Thus, one of the solutions most commonly used for water cultures, Knop's solution, shows the following composition: $Ca(NO_3)_2$, four parts; KH_2PO_4, one part; $MgSO_4$, one part; KCl, half a part; and a small quantity of $FePO_4$; in 4,000 parts of water. Recently, the American investigators, Shive and Tottingham, have used a still simpler solution consisting of three salts: KH_2PO_4, $Ca(NO_3)_2$, and $MgSO_4$. The importance of the separate elements composing the nutrient solutions will be discussed later. In this place we wish only to mention that with due care the plant thrives just as well in such a solution as in the most fertile soil and produces excellent yields (Fig. 1).

If from the number of elements composing the nutritive salts one is excluded, for instance, K, N, or Mg, the plant will not develop, or, more exactly, it will develop only as much as the reserves stored in the seeds will allow. Wherefrom it may be concluded, that all these elements normally must be absorbed from the soil. But carbon is not among these elements, though,

as far as weight is concerned, it constitutes almost half of the dry matter of every plant, including those grown in water cultures. It must be concluded, therefore, that plants do not obtain carbon from the soil and that the carbon compounds of the soil are not necessary for its development. On the other hand, it is not difficult to be convinced that carbon dioxide, which is present in the atmosphere and which constitutes on an average but 0.03 per cent of its volume, is indispensable for the nutrition of plants. For this purpose, all that is necessary is to place the plant in an atmosphere which is freed from carbon dioxide, for instance, under a bell jar, into which the air enters through a layer

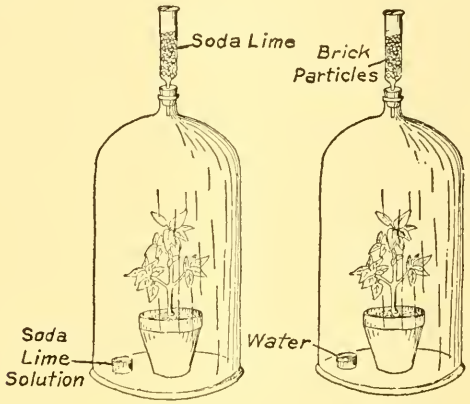


FIG. 2.—Culture of a plant in an atmosphere free from carbon dioxide (*after Smith, et al.*).

of soda lime (Fig. 2) which absorbs carbon dioxide. It will soon become apparent that the further accumulation of matter is stopped and that the plant is beginning to show signs of starvation. This starvation will be observed no matter whether the roots of the plant are in an artificial nutrient solution devoid of carbon compounds or in a soil rich in humus. In the latter case, however, measures should be taken to prevent the carbon dioxide, which is constantly liberated by the soil as a result of bacterial processes, from penetrating into the bell jar, which covers the aerial parts of the plant. For this purpose, a vessel with potassium hydroxide may be placed near the plant.

Water cultures, as well as experiments of growing plants in air free from carbon dioxide, agree that the carbon require-

ments of the plant are supplied by the carbon dioxide of the atmosphere and that the organic substances of the soil play no important rôle in carbon nutrition.

The absorption of carbon, which is necessary for the structure of the plant, from the carbon dioxide of the air, is one of the most important processes of plant life, as well as of the whole organic world. Since animals as well as man lack the power of utilizing the carbon dioxide of the air, they can obtain organic compounds only after these have been manufactured by plants. Hence, the above-mentioned process justifies a close and detailed study.

In order that the plant may absorb from the air the carbon dioxide necessary for its nutrition, certain external conditions are required. In the first place, the plant must receive sufficient light. In the dark winter season, or in the depth of a room far from a window, water cultures fail. Exact weight determinations have shown that under such conditions the plant not only shows no increase in dry matter, but actually loses weight. Another essential factor is the green color of the plant. Plants that are not green, as for instance fungi or some parasites as *Orobanche* or *Lathraea squamaria*, are unable to utilize carbon dioxide. The same is true of the non-green parts of the plant, such as the roots which receive all their food from the leaves. The green leaves, therefore, must be regarded as the special organs of plant nutrition as far as the carbon dioxide of the air is concerned.

2. Methods of Detecting and Studying the Process of Nutrition with Carbon Dioxide. Liberation of Oxygen by the Plant.—At the present time, we not only have at our disposal numerous methods by means of which we are able to detect the process of assimilation of carbon from carbon dioxide by the plant, but also we have methods to study this process quantitatively. The principle of these methods is as follows:

In order to determine the amount of carbon dioxide which is assimilated by the plant, the following procedure is used: Through a jar, containing the plant, an air current is passed in which the amount of carbon dioxide has been determined beforehand. The non-utilized portion of carbon dioxide is then absorbed by an alkali. This method shows that in bright light a branch of a plant is able to absorb almost all of the carbon dioxide from the passing air, if the current does not move too fast. Boussingault

was the first to employ this method some hundred years ago. In recent times, it has been considerably improved by other investigators. This procedure is expedient, as it allows one to observe the plant in its natural surroundings and thus to study the absorption of carbon dioxide from unaltered air.

Numerous other methods are based on the fact that in the process of nutrition with carbon dioxide the plant does not confine its activities solely to the absorption of this gas from the surrounding air, but that it releases also an equal amount of oxygen. "The plant improves the air" was the somewhat ingenious definition of the above process during the first years following its discovery.

The release of oxygen may be detected by several means. The simplest procedure is to use plants that are submerged in water to which a certain amount of carbon dioxide has been added. When such plants are exposed to a sufficiently bright light, for instance, direct sunlight, or a strong electric light of several hundred candlepower, then on the surface of the plants will appear gas bubbles. When these bubbles are gathered by means of a funnel into a test tube or eudiometer, it will not be difficult to convince oneself that the gas is oxygen. For this purpose, it is sufficient to introduce into the tube a glowing splinter. This experiment is most easily conducted with water plants. It may be vividly demonstrated with a branch of *Elodea canadensis* with the cut end turned upwards. When placed in light, air bubbles will rise with great regularity from the cut intercellular spaces. In counting the number of bubbles which are given off during 1 min., changes in the rate of carbon dioxide absorption may be observed. This method is not quite exact, since, owing to diffusion, other gases, such as nitrogen and carbon dioxide, may be dissolved in water and will mix with the released oxygen. The method most commonly applied in recent years, therefore, is an exact quantitative determination of the oxygen eliminated by the plant, by means of

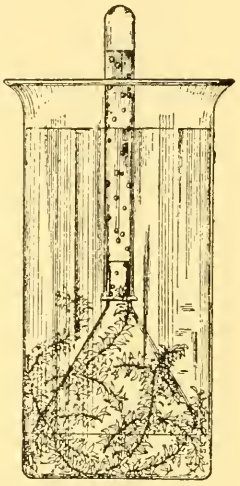


FIG. 3.—Method of gathering oxygen given off by an aquatic plant (*after Palladin*).

titrating a definite volume of water with oxygen-absorbing substances (Winkler's method).

The most complete idea of the process of assimilation of carbon dioxide is obtained by using procedures in which not only the amount of absorbed carbon dioxide is determined, but that of the released oxygen as well. The method most commonly used is one in which the leaf of the plant is placed in a eudiometer, which is closed by mercury and filled with air whose carbon-dioxide content has been determined beforehand (Fig. 4). The eudiometer is then exposed to light. After some time, analysis is made of the contained gas. By comparing the amount of the CO_2 eliminated with the amount of oxygen accumulated in the eudiometer, a complete idea is obtained as to the relative changes which take place in the composition of the air, resulting from the activity of the plant. Originally, the analysis of gas was made in the eudiometer itself, by means of adding first potassium hydroxide, which absorbs CO_2, and afterwards pyrogallol, which in an alkali solution readily absorbs O_2. At the present time, we prefer to take from the eudiometer small samples of the gas and to analyze these in a special apparatus, which allows one to determine with sufficient accuracy (up to one-hundredth of 1 per cent) the composition of as small a volume of gas as 1 cc.

Fig. 4.—Eudiometer for the study of photosynthesis (*after Kostytschev*).

A somewhat indirect, but nevertheless very important, method of determining the assimilation of carbon is the so-called dry-weight method devised by Sachs. This determination is based on the fact that as a result of the accumulation of the products of assimilation in the leaf blade, it becomes heavier, and this increase in weight may be ascertained by means of accurate analytical balances. It is necessary, however, to weigh not a fresh leaf, but a dried one, as in a recently cut leaf the fluctuation in the water content disguises the variation in the amount of dry matter. Because a dried leaf does not assimilate, it is necessary to resort to a somewhat roundabout way to ascertain the added weight of dry matter. A leaf showing as far as possible a symmetric structure

is taken for the experiment. One-half of the blade is cut away along the midrib. From this half a portion of a precisely defined area is cut out, dried, and weighed. By dividing the weight by the area, the initial dry weight of a surface unit of the leaf is obtained. The other half of the leaf blade, remaining in connection with the midrib, is exposed to light. After several hours, the weight of a surface unit is determined in the same way. The increased weight in comparison with the first half gives the increase in dry matter per surface unit.

The determinations made by Sachs, according to this method, have shown that a sunflower leaf in bright light accumulates in 1 hr. 0.914 g. of dry matter per square meter; a pumpkin leaf accumulates 0.680 g., a leaf of rhubarb, 0.652 g. The exact values of assimilation, however, must be considerably higher, as two other processes, leading to the loss of dry matter, are going on simultaneously in the leaf. These processes are the translocation of the substances which are produced in assimilation from the leaf to the stem, and respiration which is a continuous combustion of organic matter. If this loss of substance, during the experiment, is taken into account, by placing in the dark a second leaf similar to the first one, and then determining the loss in dry matter per surface unit, the values of assimilation will prove to be considerably higher. Sachs has determined the total dry weight of the organic matter, formed in an hour per square meter, as 1.7 to 1.9 g., for the sunflower leaf, and as 1.5 g. for the pumpkin leaf. These experiments have been repeated by other investigators with similar results. Hence, we may accept, as average values at least, that in 1 hr. of a summer day the plant assimilates 1 g. of dry matter per square meter of its surface.

Besides these principal methods of studying the absorption of carbon dioxide by the plant, there are other methods of a more special character. Some of these will be mentioned farther on.

3. Products of Carbon Assimilation. Connection with the Assimilation of Elements of Water.—The carbon dioxide entering the plant is completely utilized. One of the end products of this utilization is oxygen, which is given off by the plant to the surrounding atmosphere. An exact determination of the volumes of carbon dioxide absorbed and oxygen eliminated shows that these volumes are precisely equal. On the basis of this equal volumetric ratio, it may be said that the plant splits up the carbon dioxide,

releasing the oxygen, but retaining the carbon. Therefore, this activity by the plant is very frequently spoken of as decomposition of carbon dioxide instead of assimilation of carbon.

In reality, however, the process is much more complicated. No direct decomposition of carbon dioxide to carbon and oxygen takes place in the plant, as the free carbon is very inert and, once having formed, would not be able to undergo any further transformation. Simultaneously with the assimilation of carbon in the green leaf, the assimilation of the elements of water also takes place. It is not free carbon that accumulates in the leaf, but the products of its combination with hydrogen and oxygen of the water molecule, forming various carbohydrates, such as the different sugars and starch.

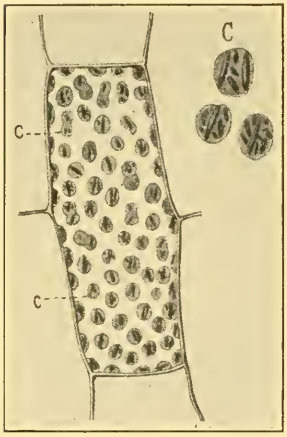


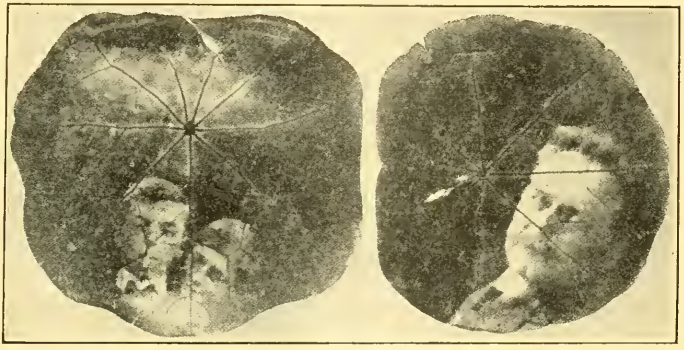
Fig. 5.—Starch grains in the chloroplasts of Elodea (*after Molisch*).

Thus by the process of the assimilation of carbon and the elements of water, 90 per cent or more of all the dry substance of a plant is formed. The process, therefore, is very aptly called "assimilation"—a term that is justified, since the assimilation of carbon, hydrogen, and oxygen is closely connected with the assimilation (*i.e.*, the formation of organic compounds) of other elements (nitrogen, sulphur, phosphorus, etc.) constituting the living matter.

Starch, the ultimate product of carbon assimilation, may be readily observed in the plant through the microscope in the form of grains. It is easily detected also by means of the well-known iodine reaction, which stains it a deep blue. The formation of starch grains in the green plastids of plants may be observed under the microscope, especially in the thin leaves of water plants (Fig. 5) and mosses or the cells of algae. With sufficient illumination, starch formation may go on even under the microscope, and the growth of the grains in the chloroplasts may be distinctly seen. Under direct sunlight, 5 min. are sufficient for the formation of starch. Even with the comparatively weak light of a kerosene lamp of 100 candlepower, Famintzin, as early as the

'sixties of last century, observed the formation of starch in the cells of *Spirogyra*, 30 min. after the beginning of the experiment.

A convenient method for detecting the accumulation of starch is the well-known Sachs test. A leaf, having been exposed to light, is decolorized by means of alcohol and afterwards treated with iodine solution. If separate areas of the leaf are shaded, prior to the experiment, with opaque paper, there will be produced light figures on the general dark background of the leaf. The relationship between the amount of transmitted light and the accumulation of starch is so close that by means of this method it



DIG. 6.—Prints from negatives on leaves made by means of a starch test (*from Molisch*).

is possible even to print on the leaves portraits from negatives just as on a sensitive paper (Fig. 6).

Starch is the most easily detectable product of assimilation. This does not mean, of course, that it is the first product, for it represents a too complicated structure made up of very large molecules. Its formation in the leaf is preceded by different kinds of sugars. Exact quantitative determinations show that not over 30 to 50 per cent of the products of assimilation is deposited in the form of starch, the rest being sugars. In many plants, for instance the onion, tulip, *Arum*, etc., the process of assimilation terminates with the formation of sugars. Such plants are called "saccharophilous," in distinction from the usual "amylophilous ones," *i.e.*, plants accumulating starch. The accumulation of starch must be regarded as a secondary process, the purpose of which is to withdraw as soon as possible the soluble

products, sugars, from the sphere of the primary assimilative reaction, since the accumulation of soluble end products always checks the progress of the reaction.

The accumulation of starch has no direct connection with the process of carbon assimilation. Experiments have shown that it may go on in leaves at the expense of sugar obtained from outside, for instance, when leaves are placed in a sugar solution and kept in a dark room.

The first product of assimilation therefore is not starch but sugar, most probably some hexose. Hence, the reaction of assimilation may be written as follows:

$$6CO_2 + 6H_2O \rightarrow C_6H_{12}O_6 + 6O_2$$

This reaction, of course, is but a scheme, because the process of transformation of carbon dioxide and water into carbohydrates passes through several intermediate reactions. These reactions have not been sufficiently determined as yet. The theories referring to them will be examined further on, after the internal and external conditions of assimilation have been studied.

The accumulation of organic substance, sugar or starch, in the process of assimilation, represents but one side of this most important process. The organic substance obtained may be subjected to combustion, in which a certain amount of energy is eliminated. Thus energy is obtained from the substance accumulated by plants in heating stoves with wood. In using vegetable products for food, they are oxidized within the body in the process of respiration and energy is obtained from them. This energy is used in maintaining the temperature of the body at a certain level (about 37° C.), as well as in work done. It should be said, therefore, that assimilation is not only a process of accumulation of organic matter, but a process of accumulation of potential energy in the form of combinations of carbon and hydrogen capable of being oxidized. This potential energy is accumulated from the radiant energy falling on the leaf, which is caught by the chlorophyll and transformed from radiant into chemical energy. The thermodynamics of the assimilation process, which plays such an important rôle in the general transformation of energy on the surface of the earth, will be discussed with more detail in Art. 8. An idea of the potential energy accumulated by the assimilation process can be given by the immense reserves

deposited in the crust of the earth in the form of peat and coal. These reserves are the chief motive power of the world's industry.

4. The Green Plastids as Special Organs of Carbon Assimilation.—The assimilation of carbon dioxide takes place in definite parts of the cell, namely, in the green plastids. An indirect proof of this is the fact that only the green parts of the plant are able to absorb carbon dioxide and to liberate oxygen. A direct proof was obtained by Engelmann by means of a very sensitive bacterial method for detecting oxygen. Many bacteria show mobility only in the presence of oxygen, even in small quantities, and possess the faculty of moving towards the source of oxygen. If a unicellular alga is placed in a drop of liquid containing such bacteria and the preparation is exposed to light, all the bacteria will gather around the alga as a source of oxygen. Care must be taken, however, to coat the edges of the cover glass with vaseline in order to prevent the diffusion of oxygen from the surrounding air. If instead of a single-celled alga, a thread of *Spirogyra* is studied, the chloroplast of which has the form of a spiral band with sufficiently large portions by hyaline protoplasm between the coils of this spiral, an accumulation of bacteria will be observed only near such parts of the cell wall that are contiguous to the spiral chloroplast. The movements of bacteria will be observed near separate chloroplasts which have been singled out from destroyed cells, though, in this case, the work of chloroplasts continued but a short time, after which they die.

Plastids without green color, as the colorless leucoplasts and the brightly colored yellow and orange chromoplasts (especially numerous in fruits and petals of flowers) do not eliminate oxygen and, consequently, are not able to assimilate carbon. Leucoplasts, however, often preserve the faculty of manufacturing starch from sugar. Such, for instance, are the starch formers in the cells of the potato tuber and other underground storage organs. All this serves to confirm the conclusion (Art. 3) that starch formation in the plastids is a secondary process and is not directly connected with carbon assimilation.

The two principal constituents of chloroplasts are the green pigment, which may be extracted with alcohol and is called chlorophyll, and the hyaline plasmatic base, the so-called stroma, which preserves the form and dimensions of the chloroplast after the chlorophyll has been extracted.

For the successful progress of assimilation, the harmonious and concordant work of both constituents of the green plastids, the stroma and the chlorophyll, is absolutely necessary. This may be seen from the fact that in chlorophyll solutions in different kinds of liquids—alcohol, benzine, and oils—the capacity of decomposing carbon dioxide has not been observed under any circumstances. The colorless plastids (leucoplasts) as well as the yellow and orange chromoplasts likewise do not exhibit the power of assimilation.

The importance of chlorophyll in the process of carbon assimilation will be discussed further on. It may be stated here that it renders the energy of the absorbed light effective in the decomposition of carbon dioxide. In all probability it takes part in this process also chemically, entering into combination with carbon dioxide and making it accessible to the influence of light. The primary importance of the stroma consists in it being the material basis through which the particles of chlorophyll are distributed and which, in a way not yet understood, makes their functioning possible. It is also the carrier of those ferments which split the first unstable products of the combination of chlorophyll with carbon dioxide, thus promoting their further transformation into carbohydrates. Finally, the stroma has still another important function, namely, the manufacture of starch from the sugar originating in the process of assimilation. This function is possessed not only by the green plastids but also by the colorless leucoplasts. The only difference between the two processes is that in the stroma of green plastids the starch is formed from sugar manufactured on the spot, while in the colorless leucoplasts the material which serves for the synthesis of starch is the sugar translocated from the leaves.

5. Chlorophyll. Its Chemical Composition, Structure, and Properties.—Chlorophyll is one of the most interesting substances. Its presence makes possible the highly important synthesis of an organic compound from the inorganic CO_2 and H_2O. At the same time, chlorophyll is one of the most complex of organic substances. Its structure and composition have been ascertained only recently, after the efforts of a number of eminent scientists covering a period of over 100 years. The difficulty of studying chlorophyll depends chiefly on its ready denaturation, requiring exclusive treatment with neutral liquids in order to extract it from the plant. Ethyl or methyl alcohol and acetone are the most common reagents used

for extraction, after which the chlorophyll is transferred to benzine or petrol ether to be purified.

According to the most recent investigations of Willstätter and his coworkers, the purest chlorophyll, freed from the admixture of yellow pigments, is still composed of two, though very similar substances, called chlorophyll *a* and *b*. The chemical composition of chlorophyll may be expressed by the following formulæ:

Chlorophyll *a*, $C_{55}H_{72}O_5N_4Mg$,

Chlorophyll *b*, $C_{55}H_{70}O_6N_4Mg$.

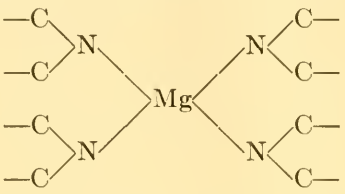
Evidently the difference between the two kinds of chlorophylls is that *b* has 2 atoms of hydrogen less and 1 atom of oxygen more than *a*. The transformation of one into the other, however, may be induced neither by oxidation nor by reduction, the difference between them thus proving of a deeper kind. They differ somewhat also in their color, chlorophyll *a* being of bluish-green, and chlorophyll *b* of a yellowish-green tinge. The amount of chlorophyll *a* is always greater than that of chlorophyll *b*. In general, for every 3 molecules of chlorophyll *a* there is present 1 molecule of chlorophyll *b*. This ratio varies somewhat in different plants, but no constant quantitative differences have been observed in various plants. The total amount of chlorophyll in a plant is not great, constituting, on an average, only about 1 per cent of the whole dry weight of the organism. In order to secure such quantities of chlorophyll as are required for exact chemical investigations great masses of plant material have to be used.

Chemically, chlorophyll may be considered a complex ester of a tricarboxylic acid—chlorophyllin—in one carboxyl of which hydrogen is replaced by the radical of methyl alcohol, in the other, by the radical of an unsaturated alcohol with a rather long carbon chain, called phytol, and of the formula $C_{20}H_{39}OH$. The third carboxyl group is connected with the nitrogen of the central nucleus forming a connection of the lactam type, *i.e.*, containing the NH·CO group. The more detailed formula of chlorophyll *a* may be represented as follows:

$$C_{32}H_{30}ON_4Mg\begin{cases}COO\cdot CH_3\\COO\cdot C_{20}H_{39}\end{cases}$$

The structure of the central nucleus of chlorophyll is of great

interest. Omitting details that would lead us into biochemistry instead of physiology, it is necessary to mention that the atom of magnesium occupies in it a central position and is connected with the atoms of nitrogen according to the formula:



The atoms of nitrogen, in their turn, constitute a part of the heterocyclic structure of the pyrrol group, which establishes a certain chemical relationship between chlorophyll and the blood pigment, haemoglobin, in whose structure the pyrrol groups also are embodied. Willstätter attributes much importance to the presence of magnesium in chlorophyll, assuming that it is joined with a molecule of carbon dioxide in the first synthetic phase of the assimilation process (see Art. 9).

The structure of chlorophyll has been established through a detailed study of the successive disruption of its molecules under the influence of alkalies and acids. The action of alkalies induces in the first place the saponification of the ester groups, *i.e.*, the splitting off of phytol and methyl alcohol, as well as the rupture of the lactam ring. The green color first becomes brown, but afterwards the ring once more closes and the green color is restored. The products of reaction are the alkali salts of chlorophyllinic acids, preserving the undisturbed structure of the central nucleus, as well as the color and fluorescence of chlorophyll. The action of acids results in the splitting off of magnesium from the chlorophyll molecule, resulting not in a fluorescent, but a brown product, which for a long time has been called chlorophyllan. This brown color is frequently observed when leaves containing considerable amount of acid in their cell sap are killed, as for instance, those of sorrel or *Begonia*, from which it is almost impossible to obtain unmodified chlorophyll. Willstätter succeeded in showing that when carefully treated with acid, the molecule of chlorophyll remains almost unmodified, hydrogen only replacing magnesium. This substituted chlorophyll he called phaeophytin. The green color may be restored if the

hydrogen is replaced by magnesium, zinc, copper, or iron. When acted upon by stronger acids, the saponification of the other groups and the further breaking down of the complex molecule of chlorophyll take place.

It is interesting and important to note that having investigated over 200 species of plants, Willstätter has found in all of them the same type of chlorophyll. Hence, this most important substance seems to be uniformly the same in all plants. From this the conclusion may be drawn that the fundamental chemistry of the process of carbon assimilation is also similar in all chlorophyll-bearing organisms.

6. Pigments Accompanying Chlorophyll.—Besides chlorophyll, the green plastids of the leaf contain two other pigments—carotin and xanthophyll. Both of these are yellow in color. They may be extracted, together with chlorophyll, by treating green leaves with alcohol. Their presence in the alcohol extract is readily detected by shaking the solution with benzine. The chlorophyll, which is more soluble in benzine than in alcohol, accumulates in the upper benzine layer, while the xanthophyll remains in the alcohol, coloring it yellow (Kraus' reaction). A complete separation of the pigments, however, is not obtained, as the carotin passes over into the benzine along with the chlorophyll and must be isolated in a much more complicated way.

Carotin is an unsaturated hydrocarbon of the formula $C_{40}H_{56}$. It is readily oxidized in air. Xanthophyll contains 2 atoms of oxygen and has the formula $C_{40}H_{56}O_2$. In its composition it is similar to carotin, although it is not simply a product of its oxidation.

These pigments, as well as the related carotins and xanthophylls, give color to the chromoplasts in fruits and in flower petals. Lutein, the pigment of the yolk of the hen's egg, is closely related to xanthophyll and is isomeric with it in its formula. The color of the tomato is produced by lycopin, an isomer of carotin.

The physiological importance of the yellow pigments of the chloroplasts is not yet understood, but, according to present information, they do not take a direct part in carbon assimilation. Some authors assume that the yellow pigments, which are always present in the chloroplasts, act as screens or light filters, thus protecting the chlorophyll against rapid destruction by light. Others believe that the yellow pigments are the carriers of the

oxygen formed in assimilation, taking it from the peroxide-like combination of chlorophyll with carbon dioxide (see Art. 9), which forms during the early stages, and afterwards giving it up to the atmosphere, thus promoting photosynthesis. These theories, however, have not been sufficiently established.

The green color of the chlorophyll is always present in the plastids of the blue-green, red, and brown algae, but it is disguised by the presence of special pigments. As these are soluble in water, they may be washed out from the dead cells of these plants, which then turn green. The bluish color of the *Cyanophyceae* is produced by phycocyanin. The color of the red algae is due to phycoerythrin, a pigment showing many modifications, wherefore the red algae are of different hues, while the color of the brown algae is induced by the presence of fucoxanthin. Our knowledge of the chemical nature and the physiological importance of these additional pigments in the algae is still very meager. Some authors, like Engelmann, assign to them an active part in the process of assimilation. This will be discussed in Chap. VIII.

7. Conditions of Chlorophyll Formation. Etiolation and Chlorosis.—The formation of chlorophyll is very little understood at present. It is known, however, that the process is conditioned by several quite definite requirements. Of these one of the most important is the presence in the cells of plastids, which can change to a green color. Chlorophyll is never formed in the roots, excepting in plants that have aerial roots, such as tropical orchids. Another important requirement for the formation of chlorophyll is light. Leaves and stems developed in darkness remain almost white or are colored yellow by carotin and xanthophyll. Such plants are said to be etiolated. They turn green rapidly when exposed to light. It is supposed that they contain a special substance called "leucophyll," which is colorless in itself, but readily changes into chlorophyll under the influence of light. The nature of this chromogen (as substances easily turning into pigments are called) has not been investigated. In some cases, for instance in the germinating seeds of conifers, chlorophyll forms also in the dark.

Beside their pale hue, etiolated plants differ from normal ones also in respect to their form. The aerial organs usually grow out very long and may be somewhat thickened, while the leaves, on the contrary, are undeveloped (Figs. 7 and 8). This modification

in form has no direct connection with the absence of chlorophyll but is due to the direct influence of darkness on the growth of cells and tissues. This phenomenon will be discussed in detail in Part IV of this book.

Another important requirement for chlorophyll formation is the presence of a minimal amount of iron salts in the cells that

FIG. 7. — Seedlings of horse beans grown in light (*from Nathansohn*).

FIG. 8. — Seedlings of horse beans grown in darkness (*after Nathansohn*).

manufacture it. Plants grown in certain water cultures, in solutions carefully purified of all traces of iron, are colored pale yellow. Under these circumstances, the process of assimilation goes on feebly, or not at all, and the plants finally die from exhaustion. But, if the leaves of such plants are touched with a solution of an iron salt, green spots are produced in places where the salt has penetrated. Localized green color may also be produced when some roots of a water culture are separately supplied with iron.

When thus treated, parts of the plant drawing directly on these roots will turn green.

The necessity of iron for the formation of chlorophyll was for a long time regarded as a strong proof of the fact that iron is one of the constituents of chlorophyll. The exact analyses by Willstätter have shown that this view is not correct. The iron ions must be regarded, therefore, as specific catalyzers without which some of the preliminary stages of the production of green pigment cannot go on, as the formation of leucophyll, for instance.

Chlorosis is sometimes observed under natural conditions, most frequently on soils rich in lime and giving a somewhat alkaline reaction. Evidently, this may be explained by the fact that under these conditions the iron salts are insoluble and cannot be absorbed by the roots. Some plants, for instance, lupins and tobacco, are very much subject to chlorosis on such soils. Chlorosis may be induced also by other causes which check the normal supply and translocation of mineral salts in the plant. Thus, in some southern crop plants (tea, pomegranate) chlorosis is observed in the spring. It is induced by the low temperature of the soil, which inhibits the activity of roots.

Chlorosis is produced also in cases of some infectious diseases. Sometimes it is localized, when the leaves show green as well as colorless portions, as in mosaic diseases of tobacco, potato, etc.

The phenomenon of chlorosis must not be confused with albinism. The latter is observed in cases where a plant, due to some internal causes, is not able to produce chlorophyll even under the most favorable external conditions. Frequently among the seedlings of corn and of other cereals, entirely white specimens are observed, which, of course, cannot assimilate carbon dioxide and therefore perish from starvation after the development of two to three leaflets. Other plants may show partial albinism when some of the leaves, or separate portions of the leaf, may be white. Such plants with variegated leaves are much valued in decorative horticulture. In the colorless portions of such leaves usually an increased amount of oxidizing enzymes will be found. But whether this increase in oxidation is the direct cause of the inability to accumulate green pigment, or whether it is but an accompanying phenomenon, is not yet clear. Variegation and albinism are hereditary characters. In crossing plants possessing these characters with normal ones, mendelian segregation is observed.

8. The Importance of Light for Carbon Assimilation. Photosynthesis and Chemosynthesis.—The mere presence of green plastids does not assure carbon assimilation. They alone cannot separate oxygen from carbon dioxide. Light is an essential condition for this process. As early as 1779, Ingen-Housz definitely established that only in the presence of light do plants "improve the air." The importance of light consists in the fact that it is a source of energy and is necessary for breaking the strong linkage between oxygen and carbon. Light is also required for the production from the highly oxidized carbon-dioxide substances of the type of carbohydrates, which on combustion can liberate a considerable amount of energy. Thus 1 g. of glucose produces on combustion 3.76 Cal., 1 g. of starch, 4.1, and 1 g. of protein, 5.7. Approximately, the same value—4.4 to 5.2 Cal.—is produced by the combustion of 1 g. of dry plant substance. In Part III it will be shown that this process of the combustion of carbohydrates and other substances of the plant body is the source of energy for the plant. If these processes were used as the source of energy for assimilation, then the plant, in order to create a unit weight of starch, would have to spend at least more than a unit weight of carbohydrates and would thus work not with a profit but with a loss, and, instead of increasing, would lose in weight. To accumulate organic compounds through the union of carbon dioxide and water, therefore, the plant requires a supply of energy from outside and receives it in the form of light, by means of which the synthesis of carbohydrates takes place. This process has been called "photosynthesis," that is, synthesis using the energy of light.

Photosynthesis is not the only way of creating organic compounds. There are plants belonging to the group of bacteria which use oxidation processes as the source of energy for the synthesis of organic compounds. In these instances, not the substances composing the body of the bacteria, but substances from outside serve as fuel. Examples of such microörganisms are the nitrifying bacteria which oxidize ammonia into nitrous acid and then into nitric acid; other bacteria oxidize hydrogen sulphide into sulphuric acid; still others, hydrogen into water, etc. They avail themselves of this energy of oxidation for the reduction of carbon dioxide and the manufacture of substances building up their body. Such a synthetic activity, based entirely on the trans-

formation of one kind of chemical energy into another, is called "chemosynthesis." These bacteria will be discussed in detail in Chap. VIII, where other oxidation processes in plants are considered. Though these bacteria, especially the nitrifying ones, are of common occurrence in nature, they constitute but a small part of the total mass of organic matter. Therefore, it may be said with certainty, that almost all of the organic matter found on the earth's surface has been created by means of radiant solar energy.

9. Relation of Chlorophyll to Absorption of Light Energy. Optical Properties of Chlorophyll. Internal Chemistry of Photosynthesis.—To be utilized in the process of photosynthesis, the

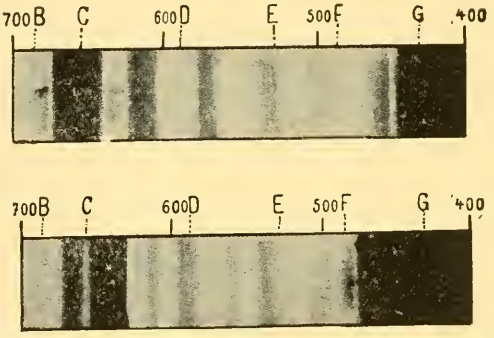


FIG. 9. — Absorption spectrum of chlorophyll. Above, chlorophyll, *a*; below, chlorophyll, *b* (*after Willstätter and Stoll*).

energy of light, in the first place, must be absorbed by a screen at least partly opaque and afterwards transformed into chemical energy. Chlorophyll is a substance with such a property. It absorbs light, not as a whole, however, but selectively. When the solar spectrum is passed through a chlorophyll solution, it is seen that separate portions of the spectrum are absorbed by it, while others are transmitted, though weakened in different degrees. In this manner the absorption spectrum of chlorophyll is obtained (Fig. 9).

The most intense absorption takes place in the region of the red rays, between the Frauenhofer lines *B* and *C* (wave-length 730 to 650$\mu\mu$). An almost equally intensive absorption will be found in the blue-violet portion of the spectrum, to the right of line *F* (470$\mu\mu$). In addition to these, several other less distinct absorption bands may be detected. The absorption spectra of chloro-

phyll *a* and *b* differ somewhat. In *b*, the absorption in the blue-violet region covers a somewhat larger region, while in the red portion the area is smaller.

As the concentration of the chlorophyll increases, the absorption bands become broader and merge one into another (Fig. 10). Finally, there are two absorption bands: one on the right side of the spectrum, covering the red and yellow rays, the other on the left part of the spectrum embracing the blue-violet rays. The green rays and part of the red ones that lie at the very limit of the dark infra-red region can pass without being absorbed.

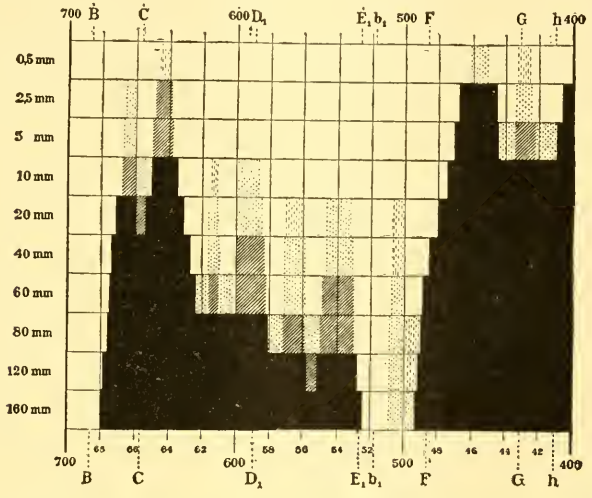


FIG. 10.—Merging of bands in the absorption spectrum of chlorophyll with increasing thickness of the solution layer (*after Willstätter and Stoll*).

It is the combination of these two spectral portions which imparts to the chlorophyll the emerald-green color peculiar to it. With further increase in concentration the green rays also are completely absorbed. Finally, only the dark-red rays remain. A very thick layer of a chlorophyll solution is no longer green but cherry red.

Besides the selective absorption of light energy, chlorophyll possesses another important optical property, fluorescence. In reflected light it appears blood red, due to the fact that a part of the rays falling on it are transformed and reflected with an altered

wave length. The faculty of fluorescence points to a considerable photochemical activity of chlorophyll.

The mechanism by which chlorophyll renders the radiant energy absorbed by it effective in the process of decomposition of carbon dioxide, is not clear to us. As has been said in Art. 4, chlorophyll plays the rôle of a sensitizer, *i.e.*, it has the faculty of rendering the energy of the absorbed rays effective for the process of CO_2 decomposition, which in itself is not subject to the influence of visible light. According to modern photochemistry, radiant energy does not move in a continuous stream but in separate jerks called quanta. Every substance subjected to photochemical decomposition absorbs the radiant energy in a strictly defined quantity—one or more quanta per molecule. The quanta are constant magnitudes for each kind of rays, but they vary with the length of the wave; the greater their length, the smaller the quantum. Therefore, the number of gram-calories of radiant energy which is absorbed in the photochemical transformation of one gram-molecule of substance is not the same for the different rays of the spectrum: the greater the length of the wave, the smaller the number of gram-calories. For rays with λ486 (blue) this number will be 58.470 Cal., for λ589 (yellow) 48.240, for λ800 (infra red) 35.510. The greater magnitude of the quanta in ultra-violet rays is demonstrated by their considerable photochemical effect, for instance, in the decomposition of silver salts in photography, and the relatively weak photochemical action of red and yellow light is due to the small size of the quanta in these rays.

The process of decomposition of carbon dioxide is, however, not photochemical in itself. By illumination [1] alone, the breaking down of the molecules cannot be induced. This is due to the fact that carbon dioxide, being perfectly transparent, absorbs almost no radiant energy, and that for the decomposition of 1 gram-molecule of carbon dioxide, according to the formula $CO_2 \rightarrow C + O_2$, 98 Cal. are required, or almost twice as much as are produced by the blue rays and three times as much as are produced by the red rays.

It must be said, therefore, that the reduction of carbon dioxide in the chloroplast is a complicated process, having besides the photochemical phase also a purely chemical one. It must be

[1] This is evidently intended to refer to the visible spectrum, since CO_2 shows an absorption spectrum in the ultra-violet.—Editors' Note.

preceded by another process going on directly in the light-absorbing molecule of the chlorophyll. The products of this process serve then for the reduction of carbon dioxide.

We are very little acquainted with the internal chemistry of the reactions taking place in photosynthesis. Until recently the most commonly accepted idea was the formaldehyde theory advanced by Baeyer in the 'seventies of the last century. According to this theory, the first product of the assimilation of carbon dioxide and water is formaldehyde, the formation of which is represented by the equation:

$$CO_2 + H_2O \rightarrow HCHO + O_2.$$

The formaldehyde obtained is polymerized under the influence of alkalies into a hexose, which was first observed by Butlerow:

$$6H \cdot CHO \rightarrow C_6H_{12}O_6.$$

Various arguments have been advanced against Baeyer's theory. The most serious of them is that formaldehyde is utilized with great difficulty by the plant for the synthesis of carbohydrates and therefore can hardly be considered as an intermediate product of assimilation. Considerations of this kind, as well as modern chemical data, have caused Willstätter to alter considerably the Baeyer's scheme. It is now supposed that the primary reaction is the union of carbon dioxide with the chlorophyll nucleus. This union of chlorophyll with carbon dioxide, absorbing directly the radiant energy, passes over into a peroxide form in the following way: .

$$RMg + HO{-}\underset{\underset{O}{\|}}{C}{-}OH \rightarrow RHMg{-}O{-}\underset{\underset{O}{\|}}{C}{-}OH$$

$$\rightarrow RHMg{-}O{-}CH\begin{cases} O \\ | \\ O \end{cases}$$

$$\rightarrow RMg + H \cdot CHO + O_2$$

where RMg, the molecule of chlorophyll, is finally restored to its original state. The formaldehyde thus originated is no longer retained by the chlorophyll molecule, but is condensed into glucose according to the above illustration.

Willstätter's theory explains clearly the catalytic action of chlorophyll, which up to this time remained somewhat mysteri-

ous. Moreover, this theory is confirmed by the fact that the dry matter of the green leaf readily absorbs carbon dioxide and is in this respect not inferior to venous blood or even alkaline solutions. Regarding the decomposition of the oxidized product with the liberation of free oxygen, this is evidently called forth by a special enzyme belonging to the group of catalases—enzymes splitting the peroxide with the formation of free oxygen.

Thus, the process of photosynthesis shows at least three phases: the union of carbon dioxide with chlorophyll, the internal rearrangement of the atoms in this compound, transforming it into an oxidized form, and the decomposition of the oxide obtained, with the liberation of free oxygen. Of these three phases, only the middle one is a photochemical process, being produced at the expense of the absorbed light energy. The two remaining phases, the first and the last, are purely chemical reactions. This complexity of the photosynthetic mechanism explains the well-known fact that its course may not be explained by the usual mechanism of photochemical reactions. This explains likewise the fact already mentioned, that solutions of chlorophyll are not able to decompose carbon dioxide, and that the assimilation process requires the presence of the whole complex:

chlorophyll + stroma + yellow pigments

The idea of Willstätter is hypothetical to a considerable degree, and is not yet commonly accepted. It must be recognized, however, as a big step forward in the study of the internal chemistry of this most important physiological process in green plants.

That the participation of chlorophyll in photosynthesis is not only of an optical but also of a chemical character is shown by the ease with which it undergoes decomposition when exposed to light. Alcohol and benzine solutions of chlorophyll, when brought into contact with air, are readily discolored by sunlight, and even by diffused light. This may be due to the acceleration of the oxidation processes under the influence of light. Chlorophyll is much more stable in the living plastids of the plant, where the photosynthetic work is going on under the conditions of strong illumination. The causes of this stability have not been discovered as yet. Some authors are of the opinion that as the chlorophyll

decomposes in the living plastid it is restored at the expense of other substances. Some suppose that chlorophyll is not in a free state in the plastids but is combined with the protein substances of the stroma, which makes it more stable. Still others assume that chlorophyll is in a colloidal condition, and therefore possesses different properties from those of a true solution. In favor of the last theory is the observation that the colloidal solutions of chlorophyll, obtained by highly diluting the alcohol solutions with water, or by triturating the leaves with water, are much more stable when exposed to light than the usual solutions in alcohol or benzine.

10. Quantitative Relations between Absorbed and Stored Energy in the Plant.—Of much importance is the question of how much the leaf profits from the radiant energy which it receives, what part of the latter is stored as potential energy of the accumulated organic compounds, and what part is utilized in other processes or given off to the atmosphere. Determinations of the radiant energy which falls on the leaf and which passes through it show that on the average about 75 per cent of it is absorbed, while 25 per cent, especially the green and red rays, are either reflected or else transmitted. From this it does not follow, however, that all of the absorbed energy is utilized by the leaf in photosynthesis. The amount of energy that is actually used may be determined in various ways. The total quantity of matter accumulated by the leaf during a certain interval of time may be determined and compared with the amount of energy absorbed during this time. The dry-weight method of Sachs, described in Art. 2, may be conveniently used for this purpose. The increase in dry weight per unit leaf area will show, as has been noted, the amount of dry matter accumulated. And, knowing the heat of combustion, it is then possible to calculate the percentage of stored energy. A number of valuable determinations were made according to this method by Krasheninnikov. Another method is to evaluate the amount of carbon dioxide decomposed by the leaf during a certain period, without injuring the leaf. Since the amount of energy required for this process is known, it is easy to compare it with the total amount of radiant energy which is absorbed by the leaf during a certain period. This method was used by the English scientists Brown and Escombe. The third method, employed by Purievitsch, is based on the fact that a leaf decomposing carbon dioxide absorbs a

greater quantity of radiant energy than a similar leaf in an atmosphere free of carbon dioxide; the difference between these values will show the amount of energy utilized in photosynthesis. All these determinations show, on the whole, concordant results, namely, that the coefficient of utilization of radiant energy by the plant has a comparatively low value. Usually from 1 to 5 per cent, and only in exceptional cases as high as 10 per cent of the whole absorbed energy is required in photosynthesis. The remaining 90 to 99 per cent passes over directly into heat energy and is utilized for the evaporation of water in the process of transpiration.

Recent determinations made by Warburg, with single-celled algae suspended in water, have given a considerably higher coefficient of utilization of radiant energy absorbed by the chloroplasts. With feeble illumination, a coefficient of 50 or even 60 per cent was obtained. This important fact shows that the chloroplast is a very perfect photochemical mechanism. If under natural conditions the leaf of a plant uses a much smaller percentage of the absorbed energy than its chloroplasts would be able to use, this must be ascribed to secondary causes, preventing the mechanism from working to full efficiency.

That the plant utilizes the photochemical capacity of the chlorophyll but insufficiently is shown by another interesting fact. The amount of carbon dioxide decomposed per unit time and per unit weight of chlorophyll is variable. In plants with dark-green leaves, this amount, termed "assimilation number," is comparatively high. Thus, for instance, in one of his experiments with etiolated bean leaves which were exposed to light and had acquired gradually a dark-green color, Willstätter observed, on the first day, an assimilation energy per unit surface of 40 mg.; on the third day, 96 mg.; and on the fifth, 104 mg. Hence, while the green color was being produced, assimilation increased 2.5 times. But the amount of chlorophyll increased during the same period 16 times, while the assimilation number was falling continually from 133 on the first day to 24 on the third and to 13 on the fifth.

Similarly, if we take different varieties of plants of the same species, distinguished from one another by darker or paler leaf color, it must not be expected that the varieties with darker leaves will assimilate more energetically than those with pale leaves, for in the pale-green leaves the assimilation number is

always higher. Willstätter observed in one of his experiments that the rate of assimilation was the same in the dark as in the pale variety of elms, though in the former the chlorophyll content was ten times greater. A similar relation was obtained by Lubimenko with shade and light plants, the first of which had darker leaves. The considerable increase of the assimilation number, coincident with the decrease in amount of chlorophyll, shows that there is always a certain surplus of chlorophyll in the leaf.

The selective absorption of light by the chlorophyll indicates that the assimilation process is not equal in the different parts of the spectrum. It has been established, long ago, that in the green rays, which are feebly absorbed by chlorophyll, assimilation is comparatively insignificant. By placing assimilating leaves under double-walled bell jars where the space between the walls was filled with colored liquids of different composition, Senebier noted, early in the nineteenth century, that in the yellow-red half of the spectrum, under a solution of potassium bichromate, the plants were assimilating more intensely than in the blue-violet half under a solution of cupro-ammonium.

The most detailed studies on assimilation in different rays have been carried out by Timiriazeff, who has proved that the maximum of assimilation lies in the red rays (between the Frauenhofer lines *B* and *C*), which are most completely absorbed by chlorophyll. The blue-violet rays, though strongly absorbed, have, however, a considerably smaller assimilative effect. This is due to the fact that they carry much less energy. And since the energy quanta peculiar to the red rays are considerably smaller, these rays, with an equal number of irradiated calories, carry a greater number of quanta and, therefore, induce the breaking down of a larger number of molecules and, consequently, also a greater energy of assimilation.

Marine algae, whose chloroplasts are not green but of other colors, show a maximal absorption of light in other spectral portions than the green leaves of land plants. According to the theory of Engelmann, these changes in absorption are connected with differences in the composition of light, resulting from its passing through the water. In sea water, which is of blue-green color, the red rays are absorbed already at a depth of 35 m., while the green rays penetrate to a depth of 350 m. Therefore, the red algae,

with a maximum absorption in the green rays, will be found at the greatest depth; the brown algae are more common at a higher level, and near the surface live the green algae, which have no additional pigments in their chloroplasts and, therefore, do not differ in their selective absorption from the land plants.

11. Leaf Structure and Assimilation. Absorption of Carbon Dioxide by the Leaf.—The process of photosynthesis takes place in the chloroplasts. Hence, in order that the carbon dioxide may serve as material for the synthesis of carbohydrates, it must be absorbed by the chlorophyll-bearing cells. These cells, forming

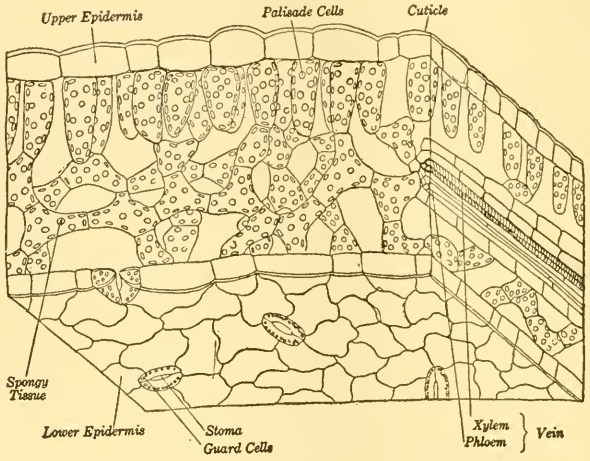


Fig. 11.—Cross-section of a sunflower leaf (*after Smith, et al.*).

the mesophyll or chlorenchyma of the leaf, are not in direct contact with the outer atmosphere, as either side of the leaf is covered by an epidermis containing no chloroplasts and, along its outer wall, with a cuticle which is little permeable to gases (Fig. 11). The only way by which the carbon dioxide is able to penetrate into the leaf and to reach the absorbing cells is through the stomata. These are small but numerous openings in the epidermis, whose structure is known from anatomical studies of plants. That the carbon dioxide enters the leaf only through the stomata may be shown by a simple experiment: If on a certain portion of the leaf the stomata are coated with vaseline, and the leaf is then exposed to light and afterwards treated with iodine, according to the

method already described, the blue-color reaction will be observed only in those portions where the stomata remained open (Fig. 12).

It is well known that the degree of opening of the stomata is not always the same. They are able to widen and to narrow their apertures or even close them entirely. These changes in the degree of opening have an influence on the process of assimilation. The closing of stomata stops it entirely. Therefore, environmental conditions that induce the closing of the stomata, usually lack of moisture, also check photosynthesis. During periods of drought plants actually suffer from starvation, they cease to develop and even lose in weight. This explains the poor development of plants in dry regions, as well as the low yields in years of drought. Assimilation is likewise suspended in evergreen plants during the cold season. Though they preserve their assimilating leaves, these organs are inactive, since in winter the stomata are permanently closed.

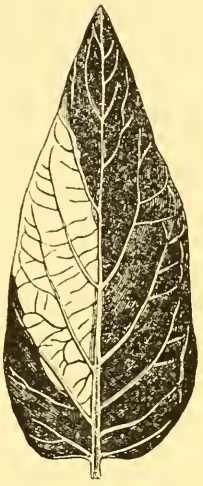


Fig. 12. — Influence of coating of stomata on the formation of starch in the leaf (*from Palladin*).

Even in the fully opened condition the stomata occupy but a small part of the leaf surface, not more than 1 per cent of the whole area of the leaf. One would think that the diffusion of carbon dioxide into the leaf must be greatly impeded, as the gas has to pass through very small openings. Brown and Escombe, however, were able to prove that an epidermis, perforated by numerous small openings, is less of an obstacle to the diffusion of carbon dioxide than might be supposed. Thus, in one of the experiments conducted with the leaf of *Catalpa*, it was observed that 1 sq. cm. of the leaf's surface absorbed 0.07 cc. of carbon dioxide per hour. An equal surface of an alkali solution absorbs during the same period from 0.12 to 0.15 cc. of CO_2, or only twice more. It must be noted, however, that of 1 sq. cm. of leaf surface only 1 sq. mm., or one one-hundredth of its area, is occupied by the stomatal openings, the remaining 99 per cent being impermeable cuticle. It may be calculated from this that the carbon dioxide penetrates into the stomata at a rate fifty times higher than the rate in entering a solution of alkali.

This seeming paradox finds its explanation in the peculiarity of the diffusion of gases through small openings. According to Stephan's law, the rate of diffusion through openings of different size is proportional to the area of these openings only when their dimensions are very large. With very small openings, the rate of diffusion is a function of their diameters.

As the number of stomata in a leaf is very large, usually several hundred per square millimeter, or several thousand per square centimeter, the sum of their diameters, in spite of their small size, is considerably greater than the diameter of the leaf. Therefore, diffusion through the membrane perforated with stomata proceeds almost at the same rate as if this membrane did not exist, and the absorbing cells of the leaf were in direct contact with the atmosphere.

In order to give an idea of the number and dimensions of stomata, the values obtained by Kiesselbach as a result of the investigation of eleven varieties of corn are presented:

Number of stomata per square centimeter of lower epidermis	7,684
Number of stomata per square centimeter of upper epidermis	9,300
Total for both surfaces	16,984
Total number of stomata per plant (average)	104,057,850
Total leaf surface per plant (average)	6,100 sq. cm.
Average dimensions of one stoma (in microns)	25.6×3.5
Average area of stomatal opening (in square microns)	89
Area occupied by stomatal openings (in percentage of total area of the leaf)	0.76 per cent

Having passed through the stomata, the carbon dioxide enters the intercellular spaces of the leaf and reaches the chlorophyll cells. In the majority of plants, on the upper side of the leaf is situated the palisade tissue, consisting of closely fitting cells which are arranged perpendicular to the leaf surface. As these cells are rich in chloroplasts, the palisade tissue must be regarded as being primarily assimilating tissue. Closer to the lower epidermis lies the spongy tissue with loosely arranged cells, forming large intercellular spaces whose dimensions frequently surpass those of the

cells. These cells contain fewer chloroplasts, and are less active in assimilation. The spongy tissue is regarded as being specially adapted to the aeration of the leaf. The stomata are usually arranged on both sides of the leaf, especially in herbaceous plants that are adapted to exposed habitats. In trees and in shade plants the stomata are situated most often on the lower side, the upper epidermis being free of them.

To secure a normal progress in assimilation, the same degree of saturation of the cells with water must be maintained. But the heat of the sun's rays induces a continuous evaporation of water from the leaf. Therefore, the leaf must have a dense network of bundles carrying water, which communicate through the petiole directly with the xylem tissues of the stem and the roots. The translocation of the products of assimilation takes place through the phloem of the same bundles, which is of great value for the unimpeded formation of new products by photosynthesis. Experiments have shown that when leaves are separated from the plant and, therefore, are unable to remove the products of assimilation, upon continuous exposure to light they will become filled with these products and as a consequence assimilation will be checked. Repletion with assimilates, as well as water deficiency in the leaves, is frequently observed during the midday hours on bright summer days, especially in dry and hot localities, for instance, in the steppe zone. During these hours, the assimilation process is perceptibly delayed, and frequently stopped. Assimilation is most energetic during the early morning hours, when the leaf still shows an abundant water supply and is at the same time free from assimilates which during the night have been translocated to other plant organs.

12. Dependence of Assimilation on Light. Light and Shade Plants. Growing of Plants in Artificial Light.—Light being the source of energy for photosynthesis, it is natural that with increased intensity of light the process also goes on at a higher rate. No direct relationship between the amount of light and the rate of decomposition of carbon dioxide, however, has been observed. If, beginning with low values, the intensity of light is augmented by the same magnitudes, assimilation will increase by progressively smaller steps till a certain intensity of light is reached. Further increase of light induces no increase of assimilation. If the dependence of assimilation on the amount of light is represented graphi-

cally (marking on one axis the quantity of carbon dioxide which is decomposed during a time unit, and on the other the intensity of light), for instance, in fractions of direct sunlight falling on the surface of the earth in the noon hours of a bright summer day, it will be seen that the curve of the dependence of assimilation on the amount of light will show, in general, a logarithmic character (Fig. 13).

Not in all plants, however, is the dependence of assimilation on the amount of light expressed by a curve of this nature. In those peculiar to open, sunny habitats, assimilation increases until the intensity of light has attained the intensity of full sunlight (the

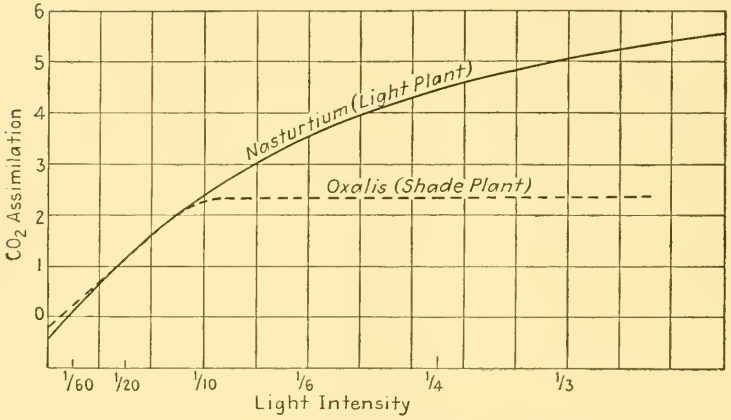


FIG. 13.—Dependence of assimilation on the intensity of light in a light plant (Nasturtium) and a shade plant (Oxalis) (*after Lundegårdh*).

continuous curve in Fig. 13). In plants adapted to shaded habitats, as for instance *Oxalis* (the broken curve in Fig. 13), assimilation increases until light has attained a comparatively low intensity, or about one-tenth of full illumination. Further increase of light remains without effect or even induces a depression of the assimilation process. In respect to their response to light intensity, all plants may be divided into two large groups, light plants or shade plants. Similar to other ecological groups, no sharp line of demarcation can be drawn between these two. They are connected by transition forms, or shade-tolerant plants.

Light plants develop the better, the more they receive direct sunlight. They will not tolerate the slightest shading and they will grow, under natural conditions, exclusively in open places.

The common weed of prairie regions, *Amaranthus retroflexus*, may be mentioned as an example of such plants. If this plant is deprived of but one-quarter of the total daylight, it is considerably checked in its development and loses its capacity of blooming. On the other hand, shade plants, for instance, *Teucrium scorodonia*, commonly found in the woods of western Europe, shows marked signs of depression when grown in open places. According to the experiments of Combes, *Teucrium* thrives when it receives but one-third of the total daylight, or under conditions where *Amaranthus* is hardly able to exist (Fig. 14).

Light plants differ from shade plants by many anatomical as well as physiological characteristics. The leaves of light plants are thicker. This is produced by the greater development of the palisade parenchyma. Frequently, in such plants, the palisade parenchyma is formed not only at the upper side of the leaf but also at its lower side. Moreover, the cells are of smaller size, the number of stomata per unit surface is considerably greater, carbon dioxide penetrates into the leaf more rapidly, and the network of conductive bundles is considerably denser. It is of interest to note that plants of the same species, when developing under different light conditions, acquire a different structure. Even leaves of the same tree will show marked shade or light characteristics (Fig. 15), depending whether they are from the north or the south exposure, from the surface or from the depth of the crown.

Shade plants differ from the light plants also in regard to the amount of chlorophyll, showing a considerably higher content in the latter. Owing to this fact, they are able to utilize the small quantities of light that have not been utilized and have been left over by the sun-loving plants. Moreover, with the same light intensity assimilation goes on in them at a higher rate than in light plants, as may be seen from the broken line in Fig. 13. But with high intensity of light, their thin leaves, which are insufficiently supplied with water by the fine network of vascular bundles, cease to increase their assimilative activity at an earlier moment, and the curve proceeds parallel to the horizontal axis of the coordinates. The chloroplasts of shade plants usually differ from those of light plants by their larger dimensions (Fig. 16).

It has been impossible, as yet, to find an exact numerical expression for the degree of light adaptation of a given plant, as in natural habitats plants develop under light conditions that vary

continuously in accord with the diurnal and annual fluctuation of solar radiation. One of the first attempts to ascertain the relative

FIG. 14.—Development of the shade plant *Teucrium scorodonia* (above) and of the heliophilous plant *Amaranthus retroflexus* (below) under different light conditions. The figures under the drawings show what part of the total daylight was obtained by the plants during their development (*redrawn from Combes*).

minimal amount of light at which the plant is able to develop was made by Wiesner. He determined the intensity of light during the noon hours in the most shaded habitats where certain

plants occur and compared it with the intensity of light in an open place. The fraction obtained shows with what part of full daylight a given plant may be satisfied and is, consequently, an indicator of their shade tolerance. The following values of the relative light minima (in fractions of full daylight) were obtained

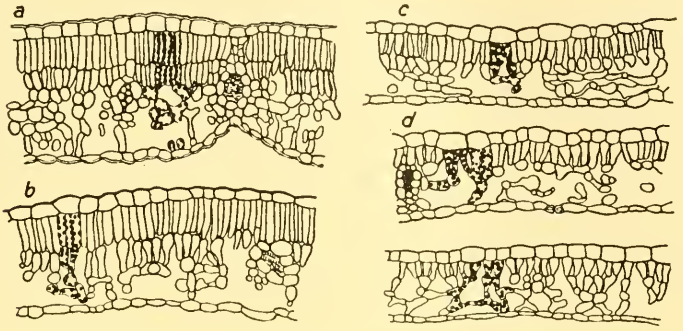


FIG. 15.—Leaves of the hazelnut: *a* and *b*, leaves exposed to the sun; *c*, *d*, *e*, shaded leaves (*after Hesselman from Lundegårdh*).

for some common trees: larch, $\frac{1}{5}$; birch, $\frac{1}{7}$ to $\frac{1}{9}$; pine, $\frac{1}{9}$ to $\frac{1}{11}$; oak, $\frac{1}{26}$; spruce, $\frac{1}{28}$ to $\frac{1}{33}$; maple, $\frac{1}{55}$; beech, $\frac{1}{60}$ to $\frac{1}{80}$; *Buxus sempervirens*, $\frac{1}{100}$. These values show that the most light tolerant of our trees are larch, birch, and pine, which like but very little shading. The woods and groves formed by these

FIG. 16.—Comparative size of plastids in sun and in shade plants: *a*, Taxus (shade); *b*, larch (sun); *c*, locust (sun); *d*, beech (shade) (*after Lubimenko*).

trees, therefore, are thin and light and the soil is covered by a thick, herbaceous growth. The spruce and the beech, on the contrary, are shade-enduring trees, and the forests formed by them are thick and gloomy. Their heavy crowns almost permit no light to pass and the soil in these woods is devoid of herbaceous cover.

The exact numerical expression of the amount of radiant energy required by the plant for its normal development will be obtained only when it will be possible to grow normal plants entirely in artificial light under controlled conditions. Several such attempts have been made (Harvey, Crocker, and Maximov). By using the light of strong electric lamps of several thousand candlepower, it is possible to grow plants and to make them bloom and mature fruits (Fig. 17) in a room entirely deprived of daylight, for instance, in a cellar. But because of the differences between the spectral composition of sunlight and that of electric light (in the light of an electric lamp there are considerably less blue-violet rays than in sunlight), the plants grown in artificial light differ somewhat in their form from normal ones. Their stems stretch too much in length, while the leaf blades remain somewhat abortive. Therefore, it is impossible to apply directly the results of these experiments to plants growing in natural conditions. Some data regarding the strength of illumination (in meter-candles) sufficient to induce flowering and fruiting of plants may be given: peas, 1,100; beans, 2,400; barley and wheat, 1,860 to 2,200; radish, 4,000; tobacco, 2,200 to 2,800; corn, 1,400 to 8,000; buckwheat, 850 to 1,100. To permit a comparison, it must be mentioned that direct sunlight, at noon, gives about 30,000 to 40,000 meter-candles. It is interesting to note that plants grown under the conditions of artificial lighting develop much better when continuously illuminated during day and night, than when subjected to an 8- or 12-hr. period of darkness. Hence, the "night's rest" is not necessary for plants. The possibility of work without rest for several months seems an amazing fact. But it must be kept in mind that in the process of photosynthesis the chief part

Fig. 17. — Buckwheat grown entirely in artificial light.

of the work is performed not by the plant but by the radiant energy.

13. Dependence of Assimilation on the Amount of Carbon Dioxide. The Carbon-dioxide Cycle in Nature.—The gradual lag in increase of the assimilation process in proportion as the intensity of light is augmented, depends not only on the fact that the chloroplasts are not able to utilize the excess of light energy, but also on the fact that the carbon dioxide absorbed by them per unit time is not sufficient for the full utilization of this energy. An increase of the carbon dioxide content in the atmosphere, there-

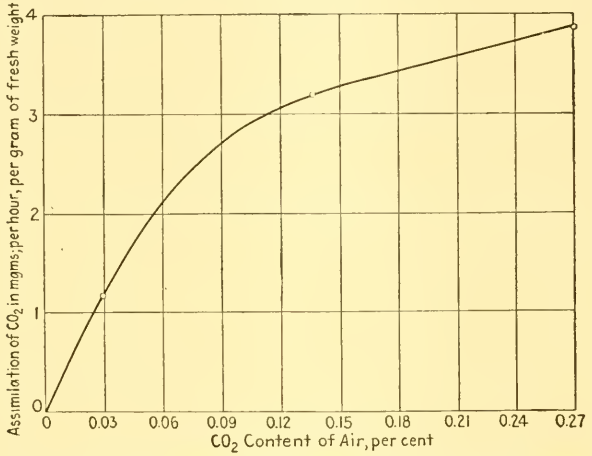


FIG. 18.—Dependence of assimilation of pine needles on the carbon dioxide content of the air (*after Stalfelt, from Lundegårdh*).

fore, always leads to an increase of the energy of photosynthesis. This may be seen from Fig. 18, showing the increase in the assimilation process of pine needles, with the increase of the carbon-dioxide contents from 0.03 per cent, the normal content of the air, to 0.28 per cent. It must be noted that the curve expressing the dependence of assimilation on the carbon-dioxide content is also of a logarithmic type, as was the curve showing the dependence of assimilation on light, and that this is the usual form of curves expressing the relationship of different physiological processes to the intensity of external factors.

Numerous investigations which have been conducted by different authorities seem to agree that the "normal" or "natural"

content of the carbon dioxide of the air, namely, about 0.03 per cent, is by no means the optimum but rather the minimum amount for the plant, and that any increase of carbon dioxide appears favorable. On the basis of the above statement, fertilization with carbon dioxide has been applied during the past few years with a view of obtaining higher yields. This has been done first in greenhouses and afterwards under the conditions of field experimentation, where carbon dioxide was conducted to the field by pipes. This method, of course, is practical only in the neighborhood of large factories, which throw into the air immense quantities of carbon dioxide (the Krupp factories in Essen, for instance, produce as much as 3,000,000 kg. carbon dioxide per day). Especially favorable results have been obtained in greenhouses where, with insufficient aeration, often not even traces of carbon dioxide can be found in the air during the day. When this gas is introduced artificially, frequently a 100 to 150 per cent increase in yield has been secured. The rapid development of the plants in hotbeds underlaid with manure seems to be due not only to the higher temperature thus produced, but also to the abundant supply of carbon dioxide.

Under natural conditions plants obtain carbon dioxide not only from the atmosphere, but also from the soil. Due to the processes of decomposition of the organic substances in the soil by different microorganisms, carbon dioxide is liberated. Diffusing from the soil in the lower layers of the atmosphere, it is caught by the leaves of the plants. According to the calculations of Lundegårdh, a sandy soil, poor in humus, liberates about 2 kg. of CO_2 per hour per hectare, while loam and clay soils containing a greater amount of humus, eliminate about 4 kg., and forest soils, extremely rich in humus, produce from 10 to 25 kg. Moderately fertilized soils produce on an average 5 kg. of carbon dioxide per hectare per hour.

When the consumption of carbon dioxide by the plant is considered with the above figures in mind, the following values are obtained. A field of 1 hectare sown to oats consumes in the process of photosynthesis about 15 kg. of carbon dioxide per hour. Five of these are supplied by the soil; the other ten are obtained from the atmosphere. In spite of the low percentage of carbon dioxide contained in the atmosphere, an air layer 100 m. thick and 1 hectare in area contains about 550 kg. of carbon dioxide. If it is assumed that in summer the most intense assimilation continues

for about 8 hr., then the oat plants will consume in the course of a day 120 kg. of carbon dioxide per hectare. Of these, 40 are supplied by the soil and 80 absorbed from the air. This constitutes but 15 per cent of the total amount contained in a layer 100 m. thick. Lundegårdh observed that during the day the carbon-dioxide content of the air is considerably lowered. At night the "respiration" of the soil once more restores the balance, producing during 16 hr. the 80 kg. of carbon dioxide lost from the air.

This balancing of carbon dioxide without profit or loss takes place only on soils of medium fertility. On poor ground, plants absorb more carbon dioxide than is lost by the soil, hence with the growth of the plants the soil becomes enriched in humus. On the contrary, in soils very rich in humus, the loss of carbon dioxide may exceed the accumulation of organic compounds by the plants. An especially great abundance of carbon dioxide has been found in forests under the cover of trees where in the lower layers of air carbon dioxide may reach a concentration of 0.08 per cent, instead of the average 0.03 per cent. This high percentage of carbon dioxide compensates the shade plants to a certain degree for the lack of light.

14. Dependence of Assimilation on Temperature. The Interaction of Several Factors in the Process of Assimilation. The Law of Limiting Factors.—Temperature is an external factor exerting a profound influence not only on all vital processes, but also on processes of a strictly chemical character. According to the well-known rule of Van't Hoff, the rate of chemical reactions approximately doubles with every increase of 10° C. in temperature. This acceleration of a reaction with the increase of temperature by 10° is termed the "temperature coefficient" and is designated by Q_{10}. Van't Hoff's rule, therefore, may be expressed by the formula $Q_{10} = 2$.

The assimilation of carbon dioxide, as well as a large number of other vital processes, is subject to the rule of Van't Hoff only within comparatively narrow limits of temperature between 0 and 30 to 35° C. With further increase of temperature the acceleration of the process is markedly delayed, after which a rapid falling off takes place, the bend in the coefficient curve often showing an acute angle (Fig. 19). With 40 to 50°, the process completely ceases. Therefore, in the temperature coefficient curve of assimilation (and other vital processes) three principal or, as Sachs called

them, cardinal points are found—the *minimum*, at which the process just begins; the *optimum*, at which it goes on at its highest rate; and the *maximum*, after which the process stops again.

According to the ingenious explanation by Blackman, the sharp bend in the temperature curve shows that we have to deal here not with one but at least with two processes. Both of them are accelerated with the increase of temperature, more or less in accordance with the rule of Van't Hoff, but inducing opposite effects in the leaf. If one of them be the process of decomposition of

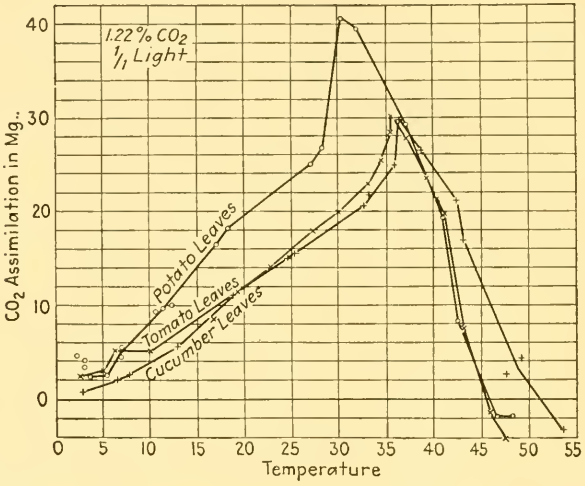


FIG. 19.—Dependence of assimilation on temperature, in the leaves of potato, tomato, and cucumber (*after Lundegårdh*).

carbon dioxide and the other the process of the "inactivation" or loss of the faculty of action of the chloroplasts, then with comparatively low temperatures the process of inactivation is almost imperceptible and assimilation is subject to Van't Hoff's law. But with a temperature of 20 to 25° the inactivation begins to tell, it proceeds at a rapidly increasing rate, and at 30 to 35° it has overcome already the process of assimilation and soon stops it. These relationships are shown diagrammatically in Fig. 20, where the curve *AB* shows the theoretical progress of the assimilation process as controlled by temperature, the curve *CD* shows the increase of the inactivation process, while the curve *AE* indicates

the actual progress of assimilation, as the resultant of the two curves.

Blackman's explanation was completely confirmed by the experiments of his coworker, Miss Matthaei. In studying the character of the assimilation curve near the optimal point, Miss Matthaei discovered that the situation at this point depends on the duration of the experiment. The longer the experiment is continued, the lower will be the temperature at which the break in the curve will occur: from 37.5° C. in an experiment lasting for 1 hr. this point is moved to 30.5° C. in one that continues for 4 hr. This is quite plausible, as during a longer period the inactivation process becomes more effective.

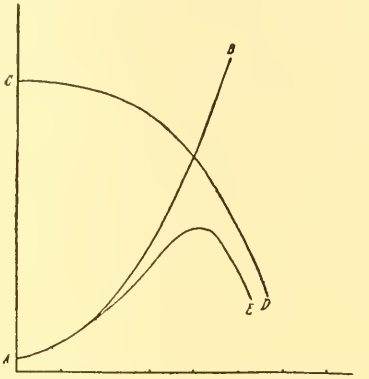


FIG. 20.—Blackman's scheme, explaining the bend in the temperature curve.

In the light of this explanation, the conception of the *optimum* does not convey the implied meaning. Literally, this term means *the best temperature*, while in reality at this point the factor of inactivation, or, more generally speaking, of the destruction of the chloroplasts, is already considerable. A somewhat lower temperature should be recognized here as the "best" at which the above factor is as yet almost imperceptible.

The influence of temperature would show itself most clearly only when the experiments were conducted with sufficiently intensive light and with sufficiently high contents of carbon dioxide. Under natural conditions these two factors, especially a sufficient amount of carbon dioxide, are seldom secured. This circumstance complicates the experiment, and the primary influence of temperature on assimilation may be entirely disguised. In studying the simultaneous effect of several environmental factors on assimilation, Blackman found that some of them, being in the minimum, limit the influence of the other factors. This dependence of one factor on another is called the "law of limiting factors."

This law operates distinctly when at least two external factors are acting simultaneously, for instance light and the carbon-dioxide content of the atmosphere. It has been pointed out that

the dependence of decomposition of carbon dioxide on each of these two factors separately may be expressed by a logarithmic curve. Suppose, now, that the intensity of the light increases, while the content of carbon dioxide remains the same, a condition that may be observed, for instance, during the day in a green house. From the moment when the intensity of light attains a level at which the carbon dioxide absorbed by the chloroplasts during a certain period is completely used up, further increase of assimilation becomes impossible, and the curve showing the

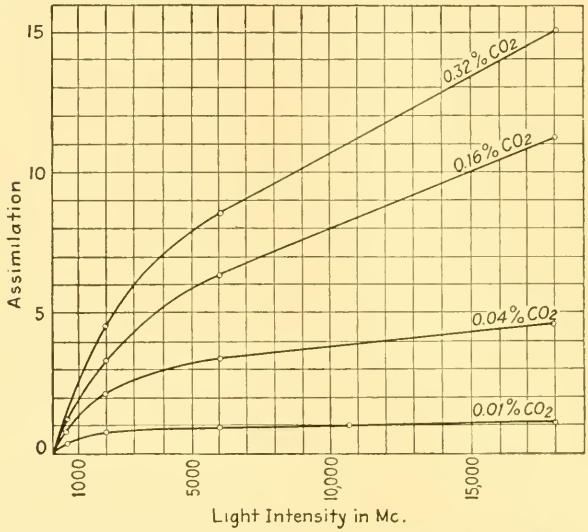


FIG. 21.—Interrelation of the intensity of light and the concentration of carbon dioxide in their influence on assimilation in the aquatic moss, *Fontinalis* (*after Harder, from Lundegårdh*).

dependence of CO_2 decomposition on the intensity of light proceeds in a horizontal direction. If the CO_2 content of the atmosphere is increased, then the bending of the curve takes place at a higher temperature than before, and only with a certain excess of carbon dioxide in the air, the actual dependence of photosynthesis on the intensity of light energy may be clearly ascertained.

Thus it is seen that a very low concentration of carbon dioxide limits the influence of gradually increasing light. On the other hand, there are conditions where too feeble light is the factor limiting the influence of the other external conditions. Hence,

the law of limiting factors may be formulated as follows: The rate or intensity of any physiological process is determined, in the first place, by the magnitude of that factor of all the external factors, which is at a minimum. In other words, this factor being in the minimum limits the influence of all other external factors.

The law of limiting factors, representing a further development of the law of the minimum established by Liebig through studies of the mineral nutrition of plants, is one of the most important laws determining the relation of the plant as a whole, as well as of its separate physiological processes, to the external environment. Formerly, it was supposed that the external factors acted separately. If one of them be limiting, an increase of the other factors will not be able to intensify a certain physiological process. Recently, however, it has been established (Benecke, Lundegårdh, and Harder) that the controlling influence of the minimal factor is not absolute but only a relative one. It slows down but does not entirely check the favorable influence of the remaining factors. This relationship is represented graphically in Fig. 21, showing the dependence of assimilation on the varying intensities of two factors—light and of carbon-dioxide content—in the aquatic moss, *Fontinalis*.

CHAPTER II

ABSORPTION OF NITROGEN FROM THE SOIL AND THE AIR

15. Sources of Nitrogen for Plants.—The amount of nitrogen contained in the dry substance of a plant is rather small—as a rule not exceeding 1 to 3 per cent. Nevertheless, it plays a very important rôle in the life of a plant. Nitrogen is an indispensable constituent of the protein molecule, which in turn is a vital part of protoplasm. Therefore, the absorption of nitrogen is by no means a less important factor in the nutrition and growth of plants than the utilization of carbon dioxide, hydrogen, and oxygen, which form 95 per cent of the dry substance.

Nitrogen is found in the environment of plants in two different forms: as elemental gaseous nitrogen, constituting almost 80 per cent of the air and as a part of various inorganic and organic substances. Gaseous nitrogen is directly accessible to the leaves and the roots of plants, since there is always a circulation of air through the soil along the capillary cavities formed between the solid particles. Excessively inundated or swampy soils contain practically no air. Nitrogen is present in the air and in the soil in the form of various chemical compounds. In the atmosphere, nitrogen is found as ammonia gas, which is produced by the decay of organic matter, and in the form of oxides of nitrogen formed by the combustion of nitrogen-containing substances and by electric discharges, as, for instance, during a thunderstorm. However, the relative amount of these forms of nitrogen is quite small. A much greater variety of nitrogenous compounds are present in the soil. Here are found both the inorganic forms of nitrogen, such as the salts of ammonium and of nitric acid, and the organic compounds of incompletely decomposed tissues of plants and animals, chiefly in the form of proteins and the products of their dissociation, the amino acids.

The question as to which compound of nitrogen may serve

as a source of this element for the nutrition of plants may be settled best by means of artificial cultures. The numerous experiments first carried out by Boussingault in the middle of the nineteenth century, and frequently repeated since, have definitely shown that molecular nitrogen, N_2, cannot be utilized by the higher plants. They cannot transform into a fixed state this highly inert gas. The compounds of nitrogen found in the atmosphere may be assimilated by plants, but their amount in general is so small that it is entirely insufficient to insure their normal development. Practically all plants, therefore, are forced to obtain the required amount of nitrogen from the nitrogenous substances found in the soil. Hence, in growing plants in artificial cultures compounds of nitrogen necessarily must be included in the nutritive mixture. The salts of nitric acid are most frequently used for this purpose.

16. Absorption of Nitrogen from Soil.—The nitrogenous substances contained in the soil may be divided into three groups: the organic compounds of nitrogen, the salts of ammonium, and the salts of nitric acid. The total amount of soluble nitrogen is quite small constituting, as a rule, not over a fraction of 1 per cent of the total dry weight of the soil. Owing to the difficulty of completely separating them from the nitrogen-free compounds, it is usually considered sufficient to determine by means of analysis the amount of nitrogen produced by the combustion of the organic substances of the soil. Hence, in reporting analytical data, the amount of organic compounds of nitrogen, as a rule, is not mentioned, but rather the total amount of nitrogen contained in these compounds. Similarly, instead of determining the weight of ammonium and nitric salts, it is usually considered sufficient to report the weight of nitrogen included in their composition. This has given rise to such terms as: "organic nitrogen," "nitrate nitrogen," and "ammonia nitrogen." These figures may be transferred into those showing the total weight of the corresponding substances, if we accept that the humus of an average soil contains approximately 5 per cent of nitrogen, potassium nitrate, about 14 per cent and ammonium sulphate about 21 per cent nitrogen.

The relative quantities of the different forms of nitrogen in various soils may be seen from certain analyses of Russian soils carried out in the laboratory of Professor Kossovitch. Generally, it was found that the black soils ("chernozem" of Voronezh

Province) contain 0.50 per cent of organic nitrogen, 0.02 per cent of ammonia nitrogen and 0.003 per cent of nitrate nitrogen; the gray forest soils (Orel Province, Russia) contain, respectively, 0.25, 0.001, and 0.0008 per cent of these substances; and the sandy soils (Leningrad Province), 0.09, 0.002, and 0.0009 per cent. These figures show that most of the soil nitrogen is found in the form of organic compounds, the inorganic ones making up but a small fraction of the total quantity present.

A further study of the question as to which compounds of nitrogen, the organic or the inorganic, are better utilized by plants, is connected with considerable experimental difficulties. Every agriculturist knows from his experience that the application of organic nitrogenous fertilizers (usually manure), markedly increases the yield. This might lead one to believe that organic compounds of nitrogen are most easily assimilated by plants. But the very fact that for fertilizing purposes it is best to use manure which is considerably decayed, suggests that the simpler compounds are better assimilated than the more complex. Experiments with soil cultures, however, so far have not been able to give a definite answer to this question. In every soil are found an enormous number of the most varied microorganisms, which decompose every organic substance introduced into a soil. The final products of this decomposition, as will be shown later, are ammonium salts and the nitrates which are always present in soil. In introducing an organic substance into the soil, therefore, it is impossible to say whether it will be utilized by the plant in an unchanged condition or whether first it will be decomposed by bacteria, and the products of its decay then absorbed by plants.

To solve this question a method of sterile artificial cultures has been devised, in which the roots of plants are placed in a medium free from bacteria. The leaves of the plants may be left exposed to the air. It has been found that, excepting a few parasitic forms, bacteria falling upon the aerial parts of a plant from the atmosphere are unable to enter plant tissues. At the present time there is a fairly large number of such methods of sterile culture (Mazè, Shulov, Petrov, Knudson, and others). Essentially, they are but variations of those used in microbiology for the culture of bacteria. The sterile nutritive medium usually is isolated from the atmosphere by means of a layer of sterilized cotton. The stem of the germinating seed is then passed through this cotton by means of

special devices, while the seed is thoroughly washed with disinfectants previous to germination, and then germination is allowed to proceed in a sterile medium. As a result, the roots of the plant develop beneath the cotton with a complete absence of bacteria, while its leaves spread freely in the air (Fig. 22).

Experiments with sterile cultures have definitely shown that many of the organic compounds of nitrogen such as amino acids, lecithin, etc., may be assimilated by plants. Their assimilation, however, proceeds at a very slow rate. Plants developing in such media are therefore considerably checked in growth as compared with plants receiving nitrates or ammonium salts. Hence, it may be concluded that mineral compounds of nitrogen are better sources of this element for plants than the organic forms, and that nitrogen in humus is utilized only after it has been transformed into inorganic form by the activity of soil bacteria. Similarly, the carbon present in humus cannot be utilized by plants until it is changed into the form of gaseous carbon dioxide, which is likewise produced in soils as a result of the life activity of microorganisms. Thus it is observed that a very close connection exists between the nutrition of green plants and the work of soil organisms.

FIG. 22.—Sterile water culture of a corn plant (*redrawn after Knudson*).

The ability to construct its body from simple inorganic substances marks a distinction between a green plant and an animal, the latter absolutely requiring organic compounds for its nutrition. Green plants, therefore, may be called autotrophic organisms. They have the capacity of independent nutrition, as contrasted with heterotrophic organisms, which are nourished at the expense of organic substances produced by other living things. In the latter group are included animals as well as non-green plants, such as the fungi and certain higher plants of parasitic nature.

17. Nitrates and Ammonium Salts as Sources of Nitrogen. Physiological Acidity of Salts.—The salts of ammonium and those of nitric acid are the inorganic compounds of nitrogen commonly found in soil. In water cultures the salts of nitric acid produce the better growth. Consequently, it was believed for a long time that nitrogen was assimilated by the plant only in this form. In cases where fertilization with ammonium salts (a sulphate is most frequently used for this purpose) produced fairly good results in soil cultures, this was attributed to the fact that ammonia is first oxidized by bacteria into nitric acid, only then becoming available to the plant.

Experiments carried out with sterile cultures have destroyed this conception. The root system of plants can absorb ammonium salts directly, providing they are in a sufficiently dilute solution. They cannot be considered a more inferior source of nitrogen for plants than the nitrates. In higher concentrations, however, ammonium salts are toxic. They cause a weaker and more irregular growth of the roots and, hence, have a harmful effect on the general development of a plant.

Besides the direct toxicity of the ammonium ion, the harmful effect of these salts is also due to the fact that their anions are assimilated by plants in a smaller proportion than the cations. They accumulate in the nutritive solution, causing its reaction to shift in the direction of increasing acidity. Thus, for instance, if ammonium sulphate, $(NH_4)_2\,SO_4$, is added to the nutritive solution, it will be observed that, as the plant absorbs the ammonium, sulphuric acid accumulates in the solution, poisoning the roots of the plant, checking its growth and even leading to its death. In general, salts whose cations are consumed by the plant with greater intensity than their anions have come to be known by the name of physiologically acid salts. Their introduction into the nutritive solution leads to its gradual acidulation, which progresses in proportion to the growth of the plant. On the other hand, there are salts whose anions are absorbed with greater avidity than are their cations. Such, for instance, is Chile saltpeter, $NaNO_3$. Its Na ion is hardly used at all by plants, while the NO_3 anion is absorbed with great rapidity. The introduction of such a salt into a nutritive solution produces a gradually increasing alkalinity. Salts of this kind are called physiologically alkaline salts. It goes without saying that this physiological acidity or alkalinity stands in no

connection with the purely chemical idea of acid and basic salts.

The harmful effect of such physiological acidity in the nutritive solution may be avoided by changing the solution frequently. In soil cultures the effect is avoided by introducing calcium carbonate (chalk or marl) into the soil, which neutralizes the free acid formed in the process of plant nutrition. Experiments carried out by Prianishnikov have shown that when ammonium sulphate is added to a soil simultaneously with chalk its fertilizing effect is no less than that of nitrates.

Ammonium nitrate, NH_4NO_3, is one of the best sources of nitrogen for plants, as both of its ions are utilized at an almost equal rate. The introduction of this salt into a nutritive solution does not cause its reaction to shift in either direction.

18. The Products of Nitrogen Assimilation and Its Connection with Photosynthesis.—As has been mentioned, the plant requires nitrogen for the purpose of forming protein substances, which are an indispensable part of the protoplasm. The proteins are the products of nitrogen assimilation. Their molecules, though, are too complex to be considered as primary products of nitrogen assimilation. It is accepted that protein formation is preceded by the formation of some simpler compounds of the type of amino-acids. Unfortunately, however, the chemistry of nitrogen assimilation is not quite so clear to us as is that of carbon assimilation.

It is easy to trace the utilization of nitrates in plants by the use of a series of color reactions. These reactions enable one to know that the nitric salts are absorbed by the root hairs, whence they penetrate unchanged into the vessels of the root system and then are transferred with water in the same unaltered state through the vascular bundles, up the stem and into the leaves. When the plant is placed into a dark room, an accumulation of nitrates occurs in the leaves, primarily in the chlorenchyma cells. This concentration is very small, however, because the ascent of water is also considerably delayed in darkness. When the plant is exposed to light, nitrates disappear from the cells of the leaf and a simultaneous increase in protein content may be observed.

The process of nitrate assimilation thus is closely connected with that of photosynthesis. It is of importance to note that, in nitric acid, nitrogen is combined with oxygen, whereas in the protein molecule nitrogen is in the form of the amino, $-NH_2$ group,

that is, in combination with hydrogen. Naturally then, the assimilation of the nitrogen from nitrates must be followed by their reduction to an amino group. The plant being exposed to light, this reduction proceeds parallel to and simultaneously with the process essential to photosynthesis, namely, the reduction of carbon dioxide.

It was at one time supposed that the reduction of nitrates was at the direct expense of light energy, and that consequently it could only occur when plants are exposed to light. Lately it has been shown that with a sufficient amount of carbohydrates present, the synthesis of proteins may also take place in darkness. This has given rise to another supposition, namely, that nitrates are not reduced by radiant energy, but through the action of the chemical energy which is stored in the carbohydrates. Hence the rôle of light in the synthesis of proteins may be an indirect one. Light is necessary for the synthesis of carbohydrates, which are consumed in the formation of protein. Which of the two ideas is the correct one has not as yet been established. At any rate, light, whether directly or indirectly, plays an important part in the assimilation of nitrates and the seat of synthesis of nitrogenous compounds is the same chlorophyll-bearing parenchyma of the leaf where the assimilation of carbon and water elements takes place.

When ammonium salts are used for the nutrition of plants, the necessity of reducing the nitrates for the synthesis of proteins is naturally eliminated. It seems, therefore, that ammonium ought to be more easily assimilated than the nitrates. But owing to its toxicity, apparently, it is not carried through the plant in an unaltered state, but is changed even in the roots into an organic substance, asparagine, and in this form is transferred to the leaves where the final transformation takes place. This rather complicated mechanism seems to encumber the nutrition of plants with ammonium salts, and thus under ordinary conditions nitrates may be considered as a better source of nitrogen for plants. The process is discussed in greater detail in Chap. VIII, where the general cycle of nitrogen transformations is considered.

19. Assimilation of Atmospheric Nitrogen by Soil Bacteria.— When the sources of carbon and of nitrogen are considered, it will be seen that in both instances green plants are obliged to utilize substances in a highly dilute state. The average concentration of carbon dioxide of the atmosphere is but 0.03 per cent, by volume,

while the average amount of nitrogen contained in soils is between 0.1 and 0.5 per cent, by weight; but, because of a high mobility of the atmosphere, the plant has at its disposal practically all of the enormous amount of CO_2 surrounding the earth. Besides, the total amount of carbon dioxide in the air is continuously augmented not only through the respiration of animals, plants, and micro-organisms, but also by the processes of combustion and by volcanic eruption. Conditions appear to be much less favorable in regard to the presence of nitrogenous substances. The amount contained in the soil is limited, and is increased only through the disintegration of such plants and animals as have existed within the same regions of the earth's surface. Moreover, a constant transformation of fixed nitrogen into free molecular nitrogen takes place in nature. This happens, for instance, when organic matter is burned; likewise, in the process of denitrification or decomposition of salts of nitric acid in the soil. Formation of free nitrogen in the soil usually occurs as a result of a superabundant accumulation of nitrogen-bearing matter. If the processes resulting in the fixation of molecular nitrogen were not present, the nitrogen balance of the earth naturally would show an ever-increasing deficit, and the organic world would be in danger of perishing from nitrogen starvation. This does not occur, owing to the fact that among the living organisms populating the earth's surface there are many that possess the capacity of carrying out the extremely difficult synthesis of organic nitrogen compounds from non-nitrogenous substances and free molecular nitrogen. These are the so-called "nitrogen-fixing" bacteria.

The majority of these nitrogen-fixing bacteria live in the soil. It has been known for a long time that fallow land becomes richer in nitrogen and that the accumulation of fixed nitrogen occurring in it is a biological process, since it is interrupted by the addition of antiseptics to the soil. Winogradsky (1893) was the first to succeed in isolating from the soil and in studying the physiology of one group of these bacteria. It proved to be a sporogenous bacillus, causing butyric-acid fermentation in sugary liquids and simultaneously fixing molecular nitrogen (Fig. 23). The discoverer has named it *Clostridium pasteurianum*. This organism belongs to the type of anaerobic bacteria. They are able to live and develop only in the absence of oxygen, and therefore always exist in soil, together with the ordinary saprophytic bacteria which

cling closely to its colonies and consume oxygen with great avidity.

The fixation of nitrogen is an endothermic process requiring an external supply of energy. Clostridium bacteria use as a source of energy the process of butyric fermentation. This mechanism will be considered in greater detail farther on (see Art. 82). Winogradsky has succeeded in establishing a definite quantitative relationship between the two processes. He found that 2 to 3 mg. of nitrogen are assimilated for each gram of carbohydrates fermented.

Somewhat later, the Dutch bacteriologist Beijerinck (1901) dis-

FIG. 23.—*Clostridium Pasteurianum.*

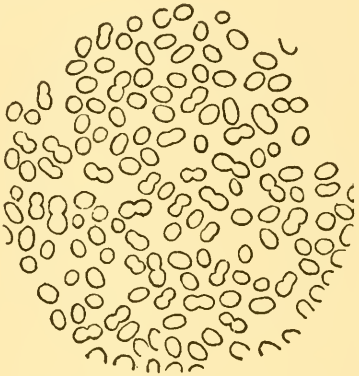


FIG. 24.—*Azotobacter chroococcum.*

covered another widely distributed bacterium, which he calls *Azotobacter* (Fig. 24). This organism has the capacity of very intensive respiration, which supplies it with sufficient amount of energy for the fixation of free nitrogen. Since a much greater amount of energy may be obtained from the same quantity of carbohydrates by the process of respiration than by that of fermentation, it is but natural that Azotobacter works with greater efficiency than Clostridium, being able to fix as much as 15 mg. of nitrogen per gram of sugar consumed. Besides these two most important nitrogen-fixing bacteria, there are large numbers of others found in soil which assimilate atmospheric nitrogen. Certain authors atttribute the same capacity even to some of the molds and blue-green algae, though this has to be confirmed.

The detailed chemistry of nitrogen fixation by these organisms is not clearly understood. There are many reasons to suppose, however, that nitrogen is combined with the hydrogen of the water molecule and that ammonia is the primary product of this synthesis. This then is utilized for the formation of the more complex substances, such as the amino acids and proteins.

Since the nitrogen-fixing bacteria require non-nitrogenous organic substances in order to obtain the necessary energy for their work, the incorporation of plant material into soil even if it is poor in nitrogen, as for instance straw, green manure, etc., considerably increases the activity of these bacteria and thus contributes to the accumulation of fixed nitrogen. A particularly intense activity is displayed by these bacteria in tropical countries, where good harvests are often secured from soils low in nitrogen and not enriched by nitrogenous fertilization. In higher latitudes, however, the activity of microorganisms is not sufficient to restore to the soil the quantity of nitrogen that is removed with the harvesting of crops. In order to enrich the soil in nitrogen, it is necessary, therefore, either to introduce nitrogenous fertilizers or to resort to the cultivation of leguminous plants.

20. Assimilation of Molecular Nitrogen by Leguminous Plants. Agriculturists have noted for a long time that the growing of legumes, particularly of clover, makes the soil more fertile, and subsequently increases the yield of cereals. It has been established also that this increased crop production results from an increased nitrogen content of the soil. Naturally, then, leguminous plants have acquired the name of nitrogen collectors. These observations, however, proved to be contradictory to Boussingault's careful experiments, which showed that under conditions of artificial culture no plants, legumes included, were able to assimilate free nitrogen of the atmosphere and that their yield was wholly determined by the amount of combined nitrogen present in the nutritive solution.

Hellriegel (1886) succeeded in solving this apparent contradiction. His attention was drawn to the fact that in soil certain nodules which were absent in water and sand cultures were formed on the roots of leguminous plants (Fig. 25). And as these nodules were found to be filled with bacteria, it was assumed that they assimilate atmospheric nitrogen, while the plant, in turn, supplies these bacteria with the non-nitrogenous nutritive materials and

then utilizes the results of their activity. This supposition was confirmed by direct experimental inoculation of sand cultures with small quantities of soil in which leguminous plants had been grown. Control plants, which had not been inoculated, did not form nodules on their roots and showed no evidence of assimilation of atmospheric nitrogen. Numerous nodules appeared on the roots of plants in the inoculated vessels and the amount of nitrogen contained in the yield was much greater than that originally introduced into the soil in the form of saltpeter, $NaNO_3$.

FIG. 25.—Nodules containing nitrogen-fixing bacteria on the roots of leguminous plants: *A*, red clover; *B*, sweet pea; *C*, soybean (*after Transeau*).

These tubercles found on the roots of leguminous plants are tumors formed as a result of an excessive growth of the parenchyma cells of the cortex (Fig. 26). They consist of large cells densely filled with bacteria. These bacteria enter the root through the root hairs (Fig. 26*B*), whence they penetrate in threadlike formation, consisting of innumerable bacteria joined together by mucilage, into the parenchyma of the root, producing a localized perforation of the cell walls and stimulating the surrounding tissues to rapid growth. The cells of the tumor become crowded with bacteria but remain alive and preserve their large nuclei. At first the bacteria nourish themselves at the expense of the host plant. They are parasites. Hence during the period subsequent to inoculation,

the growth of the plants is somewhat retarded, but the inoculated plants soon recover and begin to show more rapid progress in their development as compared with control plants. Towards the end of vegetation the bacteria contained in the cells considerably decrease in number and are changed into an irregular form (Fig. 26, *C*, *D*, *E*), the so-called bacteroids. Apparently the greater part of the bacteria die and are dissolved as a result of the effect produced on them by the living protoplasm of the nodular cells. The products of their disintegration are absorbed and assimilated by the plant. The nodules having rotted and separated from the

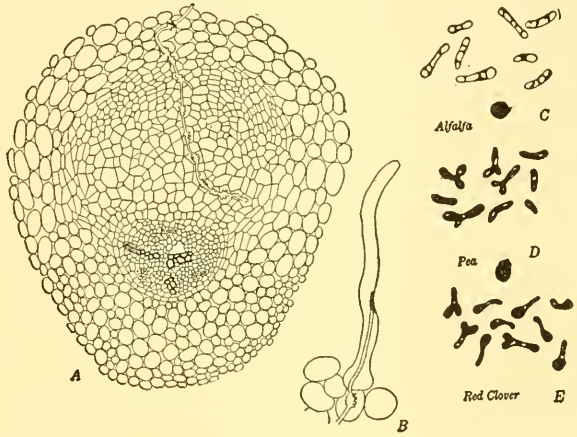


FIG. 26.—Cross-section of a young nodule of vetch, showing infection thread of *Bacillus radicicola*. *B*, infection thread, having entered the root hair; *C*, *D*, *E*, bacteroids from different plants (*after Smith, et al.*).

roots, the surviving bacteria are liberated into the soil where they continue to exist, though here they multiply more slowly than in the nodules. A new seeding of a leguminous plant in the same soil then may have its roots inoculated with the bacteria.

The relationship existing between leguminous plants and nodular bacteria is usually regarded as one of symbiosis, namely, an intimate union of two organisms, both of which are benefited. The bacteria receive carbohydrates from the plant and in turn supply the plant with combined nitrogen. This relationship, like other types of symbiosis, however, may be regarded as a sort of balanced parasitism. First, the bacteria are the assaulting party, behaving as parasites in respect to the plant and causing, as

already shown, a temporary delay in development and, in some instances, when the plant is not sufficiently strong, even its death. Later, however, the upper hand is gained by the plant, which then absorbs from the tubercles the nitrogen compounds produced by the bacteria and finally digests the greater part of them.

The bacteria found in nodules have been isolated in pure cultures and have been named *Bacterium radicicola*. Being adapted to a parasitic life in the roots of plants they do not grow readily in an artificial medium. Moreover, under artificial conditions, they show hardly any signs of assimilation of molecular nitrogen. Several races of such bacteria have been isolated, each of them corresponding to a definite group of leguminous plants, whose roots they can inoculate. Thus, for instance, the bacteria from beans readily inoculate vetches, but are ineffective on alfalfa. In introducing new leguminous plants, such as, for example, soybeans or lupines, therefore, it is necessary to incorporate into the soil cultures such nodular bacteria as are adapted to these plants (Fig. 27). Thus a proper "bacterial fertilization" often increases the yield considerably. Inoculation with nodular bacteria may be found especially useful when leguminous plants are grown on drained swamps, because these bacteria are usually absent in boggy soils. All leguminous plants possess the capacity of entering into symbiosis with tubercle bacteria, this being an important physiological peculiarity of this group of plants. It is rarely found in other groups of plants. Among the trees are found similar, but perennial tubercles on the roots of the alder and *Eleagnus*; while on certain tropical trees and shrubs, belonging to the family of *Rubiaceae*, the bacterial tubercles are found on the leaves. In these cases, *Bacterium radicicola* are not present,

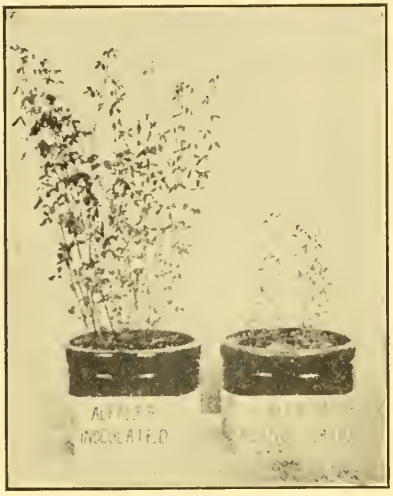


FIG. 27.—Alfalfa grown in soil poor in nitrogen: left, inoculated with specific bacteria; right, not inoculated (*after Smith, et al.*).

but other similar microorganisms are found. The physiological peculiarities which prevent the nitrogen-fixing bacteria from living on the roots of other plants are not understood as yet. Evidently here we have to deal with the same specific adaptability of organisms towards each other as is observed in most cases of real parasitism.

21. The Nitrogen Cycle in Nature. Artificial Nitrogenous Fertilizers.—The ability of leguminous plants to fix molecular nitrogen of the atmosphere is of enormous importance in the economy of nature in general and in the economy of agriculture in particular. Leguminous plants are widely distributed over the earth's surface. They are quite common in every plant association. The nitrogen which they accumulate is of such an amount that it is not only sufficient for their own nourishment and for an abundant storage in their seeds, but it also produces a general increase in the nitrogen content of the soil. This enrichment of the soil is due to rotting of fallen leaves, of nodules remaining in the soil, and of other disintegrating parts of the plant.

The capacity of leguminous plants to accumulate nitrogen in the soil explains their popularity in agricultural practice. The favorable effect of their cultivation on the subsequent yields of cereals was known long before Hellriegel's time. One of the classical experiments carried out by Laws and Gilbert at the Rothamstead Agricultural Experimental Station in England has shown that when wheat alone was grown for ten successive years on one of two identical fields and wheat in succession with leguminous plants, on the other, five yields of wheat from the second plot were equal to ten from the first. In addition, five harvests of leguminous plants had been gathered from the second plot, and each of these contained more nitrogen than in the five crops of wheat. Thus, the effect produced by leguminous plants seems to be almost miraculous. The plants themselves not only are excellently nourished, but in addition the most important nutritive element is left in greater abundance than it was contained in the soil before the experiment.

In general, plant production may be considered as a practice in which highly valuable food and technical products are created from raw materials, like carbon dioxide, water, and radiant energy, which cost practically nothing. The requisite amount of nitrogen and mineral substances, however, is often quite expensive.

Hence, the struggle for the possession of the most fertile territories of the earth is in its essence a struggle for fixed nitrogen, which most frequently is the limiting factor in plant production. The growing of legumes is a means of utilizing a free source of a very valuable substance, the molecular nitrogen of the atmosphere.

In spite of the ever-increasing intensification of agriculture, however, which is the result of an incessant growth of the world's population, the beneficial effects of leguminous plants (as well as that of free-living soil bacteria) have been found to be insufficient already and other more rapid ways of enriching soils with nitrogen must be discovered. Until quite recently almost the only method of achieving this was by the application of Chile saltpeter, enormous deposits of which have been exploited in South America for many years. The introduction into the soil of such organic material as manure, animal residue from slaughter houses, city refuse, and the like, is nothing but an incomplete return to the soil of that which was previously taken from it, as part of the nitrogen always is lost in the processes of decay and denitrification, or is carried to the ocean by rivers.

A desirable feature of potassium nitrate, when compared to stable manure, is its more concentrated form. One ton (1,000 kg.) of stable manure contains, on an average, only about 4 kg. of nitrogen. In order to obtain the same amount of nitrogen 20 kg. of potassium nitrate are sufficient. On the other hand, stable manure contains other nutritive substances necessary for the plant. Moreover, its application considerably improves the physical properties of the soil. Fertilization with stable manure is therefore one of the chief methods of improving soils poor in nitrogen, even when the so-called mineral fertilizers are extensively used.

The deposits of saltpeter, however, are being gradually exhausted. This seems to threaten mankind with the possibility of nitrogen starvation. Modern technique, however, has come to the aid of agriculture. Several years previous to the World War a method of obtaining nitric acid from the air by means of a high potential electric discharge was found. This method is being widely applied, especially in Norway, a country rich in waterfalls, which produce cheap electric energy. Hence the product—calcium nitrate—bears the name of Norwegian saltpeter. During the World War the German chemist, Haber, discovered another

method of fixing atmospheric nitrogen by combining it with hydrogen, thus forming synthetic ammonium. At the present time, there are in operation in Europe several factories which produce hundreds of thousands and even millions of tons of synthetic ammonium salts, and it is very likely that in the near future the requirements of agriculture will be fully satisfied by artificial nitrogenous fertilizers produced from the air. Then the increase of yields will not depend, as heretofore, on the growing of nitrogen-gathering leguminous plants.

CHAPTER III

THE ABSORPTION OF MINERAL ELEMENTS

22. The Ash Constituents of the Plant. Their Composition and Origin. The Indispensable and Minor Mineral Elements.— One of the principal methods of analysis of the plant consists in burning it. During this process the carbon is determined as carbon dioxide, hydrogen and oxygen in the form of water, and nitrogen in the form of molecular N. After the burning of the dry substance of a plant, there always remains a non-volatile residue, the ash. This, too, represents an indispensable part of the living substance.

The quantity of ash in different parts of a plant is variable. The least amount, about 1 per cent, is found in the wood of trees, which consists primarily of the walls of dead cells. Seeds contain about 3 per cent of ash, the main vegetative organs, stems and roots of grassy plants, 4 to 5 per cent, while the leaves may have as much as 10 to 15 per cent. It is of interest to note that a relatively high concentration, approximately 7 per cent, of ash is found in the bark of woody plants. Tissues consisting mainly of living cells are usually richest in ash. It must be understood that these are approximate figures. They will vary not only with different plants, but even within the same organs of a species the quantity of ash can fluctuate considerably. Leaves of potatoes, for instance, may contain from 5 to 13 per cent of ash; those of beets, 11 to 21 per cent; and of rape, 8 to 15 per cent. The ash content depends largely on the composition and moisture content of the soil. In general, one may say that the richer in salts the soil is and the dryer the climate, the more ash accumulates in the plant, although a direct proportionality may not always be observed.

The composition of the ash is very complex and variable. Almost all of the elements, including the rarest ones, have been found in the ash of some plant. Many of the rare elements are

dispersed in the crust of the earth in such small amounts that they may not be discovered by ordinary analysis, but they may be present in a considerable concentration in plants. Bromine and iodine, for example, accumulate in large quantities in the seaweeds. Some geologists, as for instance Vernadsky, attribute to the accumulating power of the organisms a significant rôle in the general circulation of the rare elements on the earth's surface.

The following elements are most often found in the ash of plants: K, Na, Ca, Mg, Fe, Mn, Zn, B, Al, Si, P, S, and Cl. It does not mean, however, that all of these are really necessary. It has been seen that the normal development of plants may be obtained in water cultures on four- or even three-salt solutions which contain only K, Ca, Mg, Fe, S, P, and N.

To these seven principal elements, whose necessity for the nutrition of the plant was proved in the middle of the last century by the classical experiments of Knop and Sachs, several others have been added: manganese, zinc, boron, and silica. These four elements were formerly regarded as superfluous, accumulating in the plant only by virtue of the fact that they are dissolved in the water that is absorbed by plants from the soil. This increase in the number of necessary elements was the result of water cultures conducted with salts carefully purified from the smallest amounts of impurities by the exact methods of modern chemistry, and used in containers coated on the inside with a paraffin layer, in order to prevent the solution of elements found in the glass. All of these substances, similar to iron, are required by plants only in exceedingly small quantities. With the usual method of water cultures there is no need to introduce them into the nutrient solution. Traces are always found in the common "chemically pure" reagents or are leached from the glass walls of the container. It is not improbable that further investigations with more carefully purified reagents will reveal the necessity of still other elements for the normal development of the plant, elements which now are regarded as accidental and useless admixtures.

It must be noted that the relative amount of one or another element in the ash of the plant by no means indicates the degree of its necessity. Thus, the plant can do without sodium, an element constituting a perceptible part of the ash of plants and even accumulating abundantly in some of them. On the other hand, the presence of traces of manganese and boron is absolutely neces-

sary. Though a plant always contains a considerable amount of silica in its ash, only an insignificant part of this is really necessary. The remainder is evidently useless. Sometimes, however, the accumulation of superfluous elements may play an accessory rôle. Thus, the silicic acid accumulating in the cell walls of cereals serves to stiffen them.

FIG. 28.—Water cultures showing comparative growth of plants: normal (in the center), with one of the indispensable elements absent (on the left and right) (*after Pfeffer*).

The common elements used in nutrient solutions are absolutely necessary for the proper growth of plants. The exclusion of any of them leads to a check in development and finally to death of the organism. Figure 28 shows clearly what conspicuous differences may be observed in the development of plants in a complete nutrient solution and one from which a single element is absent. It seems to make little difference which of the elements is excluded, all of them being equally necessary. The slight difference that may be observed when various elements are excluded depends not so much upon their comparative value as upon the amount of the particular element stored in the seed and upon the quantity used for the development of the plant.

23. Importance of the Different Groups of Ash Constituents. Nutrient Elements as Regulators of Vital Processes.—The conception of the value of ash elements, which originated from the results of the application of analytical methods, is from a physiological point of view quite artificial. That certain ash constituents remain after the ignition of plant material does not necessarily give an indication as to the part they may play in the living organ-

ism. The reason that this artificial grouping is retained in text-books is not due to their close relationship from a physiological point of view, but rather to the similarity of their origin. All of them are obtained from the mineral constituents of the soil. That is the reason for their being called mineral substances of the plant.

The major ash elements may be divided into two distinct groups: the metalloids, such as sulphur and phosphorus, and the metals—potassium, calcium, magnesium, and iron. Sulphur and phosphorus are found combined with the most important organic substances of the protoplasm in the proteins and nucleoproteins. As they possess a number of similar properties, S and P may be compared with nitrogen, which is also indispensable for the building of the protein molecule. The metals, on the contrary, are present in the plant as free ions. This is especially true with regard to potassium, up to 90 to 98 per cent of which is found in the plant in ionic form and may be extracted from the dry-plant substance by water, combustion not being necessary. Metallic elements play the rôle of regulators of vital processes.

This, however, is not the only function of the metals. Some metallorganic compounds are present in the most important parts of the cell, where they have a very vital function. Magnesium, for instance, is found in the chlorophyll molecule. Hence at least some of the metals may be considered as nutrient substances. Phosphorus, as phosphoric acid, on the other hand, may be found also in inorganic form. In leaves, up to 50 per cent of the phosphorus is found in the form of free phosphoric acid, and during the germination of the seeds an almost complete disintegration of all organic phosphorus compounds takes place. In general, then, the separation of the ash elements into two main groups, as nutrient substances and as regulators of vital processes, indicates more clearly the function they may assume in the life of the plant. It must not be forgotten that the same element in some combinations may be a part of one group; in other combinations, it may be in another group.

24. Sulphur and Phosphorus. Their Function in the Plant and Their Cycle in Nature.—Sulphur and phosphorus are just as important to the plant as is nitrogen. Both of these elements enter into the composition of protein substances, sulphur being found in all proteins, phosphorus only in the more complex ones—

the nucleoproteins. It is also present in the other proteins of the protoplasm that have been studied but little and often are known by the general name of "plastins." As the nucleoproteins and the plastins form vital parts of the living cell, it is quite clear that sulphur and phosphorus are necessary for the plant.

Of the numerous compounds of sulphur, plants absorb only the sulphate ion, the most important source being various sulphates, including the slightly soluble calcium sulphate (gypsum). The free sulphates enter the roots without undergoing any change. They ascend with the water stream through the vessels, reach the chlorophyll-bearing cells of the leaf, and here together with nitrogen and the carbohydrates are used in the building of protein substances. At the same time reduction processes take place, for in the protein molecule sulphur is combined with carbon and hydrogen, not with oxygen. The mechanism of the reduction of sulphur is not understood as yet.

When plant or animal tissues decay in the soil, sulphur is split from the protein molecule in the form of hydrogen sulphide, just as nitrogen is freed in the form of ammonia. But, unlike ammonia, hydrogen sulphide is not available to plants, being very toxic to the roots. It may become available, however, when oxidized to sulphuric acid through the activity of special bacteria.

These bacteria represent a special physiological group of autotrophic chemosynthetic organisms of which we have spoken already in Art. 8. Their physiology has been investigated by Winogradsky. Through the oxidation of hydrogen sulphide they secure energy, which is necessary for the decomposition of carbon dioxide, and for the building of their own substance. This oxidation is accomplished in two phases: first, hydrogen is oxidized to water, the free sulphur remaining in the form of bright drops in the cells of the bacteria. Then this sulphur is further oxidized to sulphuric acid.

Sulphur bacteria are of very great importance in nature. They are present not only in the soil, but also in the upper layers of water in ponds, ditches, and other basins, at the bottom of which organic substances decay. These bacteria utilize the hydrogen sulphide rising to the top. One may obtain an idea of their activity from the fact that at the lower depths of such large bodies of water as the Black and Azov seas the water is poisoned by hydrogen sulphide, and no animal life is possible there. The

upper part of the water, however, does not contain even traces of this gas, due to the activity of vast numbers of sulphur bacteria, which are distributed in an unbroken layer to the depth of 200 m.

Similarly to sulphur, phosphorus is available to plants only in the highest form of oxidation, namely, salts of orthophosphoric acid. It does not undergo quite such complex transformations in the plant body, however, for it is found in the same oxidized state in the proteins, which may be regarded as substitution products of phosphoric acids. Besides, in plants various esters of phosphoric acid are found. Some of these may represent the reserve products of phosphorus, as for instance phytin, $C_6H_6(OH_2PO_3)_6$, which is an ester of the cyclic hexatomic alcohol, inosite, $C_6H_6(OH)_6$, and contains 6 molecules of phosphoric acid. Like many other plant reserve products, phytin is digestible by animals. Hence it is widely used in the diet as a remedy in cases of phosphorus starvation. Another group of phosphoric acid compounds is represented by the so-called "lipoids," fatlike substances containing phosphoric acid as well as some basic nitrogen. The lecithins and phosphatides also belong to the group of lipoids. Like the proteins, they form indispensable compounds of the protoplasm. Moreover, they seem to play an important rôle in determining the osmotic properties of the cell, which we shall discuss in the next chapter.

A considerable part of the phosphoric acid, sometimes 50 per cent of the whole amount, remains in the plant in ionic form, and plays a very important rôle in the regulation of the active concentration of hydrogen ions in the plant (Art. 36). Many inorganic phosphates are found in those parts of the plant where an energetic mobilization of the reserve substances takes place, for instance, in germinating seeds.

When plants or animals decay, the phosphoric acid is liberated largely in the form of inorganic salts, which are available to plants. Complex organic substances of phosphorus cannot be utilized by plants without decomposition. This has been proved by numerous sterile cultures of plants which included such substances. If the cultures were successful, that is, if they remained sterile to the end, the plants developed very poorly. If by accident they were infected, which caused a breaking down of the complex phosphorus compounds, then the plants grew much better.

25. The General Rôle of Cations and Their Specific Activity.— The rôle of the cations in the life of the plant is more complex

and less understood than that of the anions of phosphoric and sulphuric acids which have been discussed. Usually cations are found in plants in a free state. It has been established by artificial cultures that a certain combination of cations is quite indispensable for the normal development of a plant and that none of them, neither potassium, magnesium, calcium, nor iron can be excluded from the nutrient solution nor replaced by another element.

The great advances which are being made in the study of colloidal chemistry begin to reveal the physiological significance of the cations, though we are still far from knowing this subject completely. As is shown in the next chapter, the colloidal state represents a condition of unstable equilibrium. Colloids may be regarded as heterogenous systems of fine suspensions of solids in water or of emulsions of small droplets of oil. It must be understood that the solid or liquid colloidal particles are much smaller than the particles of the solid or liquid phases of relatively coarse mechanical mixtures.

Of the forces that hold the colloidal particles suspended in the liquid, preventing their precipitation and their adherence to each other, the electric charge of the particles is of the first significance. All of them carry the same electric charge, while the suspending liquid is of the opposite charge. Hence their mutual repulsion. When an electrolyte is introduced into a colloidal solution, the electrostatic equilibrium is immediately changed, resulting either in a diminished or an increased charge of the particles and thus causing their precipitation, or an increased stability of the colloidal suspension.

All vital processes of the cell, such as the movement of protoplasm, its increase in mass during nutrition, the growth of the cell, etc., are dependent on changes in the stability of the colloidal system. Cell colloids may change from sols to gels and vice versa, as for instance during the growth of cell walls or the formation of starch grains or when chromosomes divide. Thus the electrolytes may be considered as most important regulators of many functions of the cell, and the rate and even the direction of various processes of the cell may be influenced by the composition and concentration of the salts.

Since the effect of the ions on the colloids depends on their electric charge and on their specific properties, one should not be

surprised that the presence of an intricate system of anions and cations is necessary for the complex colloidal phenomena which are part of the life of the cell.

The requirements of the cell of a definite combination of cations makes it very difficult to study the specific rôle of each of them. By excluding from the nutrient solution one or another of the elements we can only ascertain that the development of the plant has been inhibited. In an experiment with buckwheat, for instance, the following results were obtained: In a complete nutrient solution the weight of the plant was 138 times greater than that of the seed; in a solution without K — 9.2 times greater; in one without Ca — 1.3 times; without Mg — 5.1 times; and without Fe — 7.3 times. It would be a mistake to conclude from these results that the elements, the exclusion of which gave a small decrease in yield (K, Fe), are less necessary than the others. Two contributing causes may have influenced the visible effects. There might have been a greater storage of these elements in the seed or else their absence may have caused less evident disorders in the organism.

In general, it must be admitted that very little is definitely known of the specific functions of the different cations and of the various disturbances caused by their absence. The only fact established with certainty is that each of the cations has its specific rôle and cannot be substituted by another, even one closely related in the periodic system. Thus potassium cannot be substituted either by lithium or by sodium, and only a few fungi allow a partial substitution of potassium by rubidium. Calcium, likewise, cannot be replaced by any of the alkali elements, and iron cannot be substituted either by cobalt or nickel. The rôle of the various metals in the life of the plant will be discussed only in general.

Potassium is closely connected with the vital activity of protoplasm, since most of it is found in the meristem of the young organs, the cells of which are rich in protoplasm. An important rôle is ascribed to potassium in the general metabolism of the cell, especially in the formation of the carbohydrates and proteins. In the active regions of the plant as much as 50 per cent of K_2O may be found in the ash. Of all the necessary cations, potassium is the only radioactive element. Thus it may have also some other significance for the plant as yet unexplained.

The magnesium content in the ash is considerably less than

that of potassium. Both seem to be widely distributed in the various organs. Like potassium, magnesium is found mainly in the seeds and young organs, where it constitutes 10 to 15 per cent of the total ash content. Its physiological function evidently is similar to that of potassium. Besides, magnesium is present in some of the most important metallorganic compounds, of which chlorophyll is a conspicuous example. Up to 50 per cent of magnesium may be found in the combined state, while potassium is present in the plant almost entirely in the form of free ions.

Regarding the demands of calcium by the plant, one must note that for the lower organisms without chlorophyll, such as the fungi and bacteria and which in general have the same requirements for cations as the higher plants, calcium is not absolutely necessary and that calcium is abundant in the older parts of higher green plants, for instance, in the bark of trees, and in old leaves. One of its most important functions seems the neutralization of the poisonous oxalic acid, which is formed in many plants as a byproduct of metabolism. Hence, in plant tissues crystals of calcium oxalate as druses, raphides, and other formations are found. A small amount of calcium is, however, absolutely necessary for the growth of young tissues, and if calcium is excluded from the nutritive solution in water cultures, a marked reduction of the root system is observed.

Iron is necessary for the formation of chlorophyll. In its absence the plants become chlorotic. This is not the only rôle of iron in the life of the plant, however, as it is equally necessary for organisms without chlorophyll. Iron is generally accepted to be a catalyzer in the oxidation processes closely connected with growth, but especially in respiration. Manganese seems to have a similar function.

The significance for the plant of those slight traces of zinc, boron, silica, and perhaps some other elements, the necessity of which for the successful growth of plants in water cultures has been revealed after the use of reagents purified in the highest degree, remains unknown so far. Evidently these ions must be added to the number of catalysts necessary for the plant.

26. Toxic Properties of Single Salts. Balanced Solutions.— The study of the specific activity of separate cations is highly complicated by another very important factor, namely, the toxicity

of solutions containing only one of the salts used in the complete nutrient medium. If the roots of a plant are divided into four parts and each of them placed in a separate medium of the four salts that enter into the composition of Knop's solution, then, instead of developing normally, the roots would be poisoned. The same would happen if, in turn each of the salts was supplied; for instance, during the first week one of the salts, the next week another, and so on.

This poisonous effect of single salts was first observed on mussels (Ringer, 1883), and on sea animals (Loeb, 1898). Solutions of sodium chloride, corresponding in concentration to sea water, were found to have a pronounced toxicity, if very pure salts were used. But very small quantities of calcium and magnesium checked this toxic effect. In a pure solution of sodium chloride, for instance, not a single embryo developed from the fertilized eggs of the sea urchin. But the addition of 1 cc. of a very dilute solution of $CaSO_4$ caused the development of 3 per cent of embryos; 2 cc., 20 per cent; and 4 cc., 75 per cent. Pure solutions of calcium salts also are toxic, although this can be counteracted by the addition of sodium salts. Thus different salts destroy the toxic effect of each other. This phenomenon has been termed "antagonism of ions."

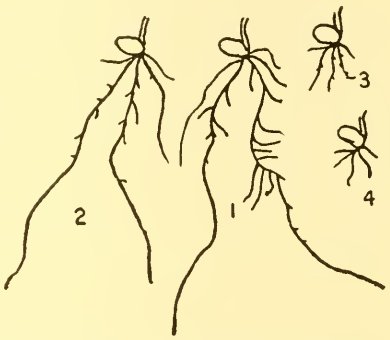


FIG. 29.—The comparative growth of roots of wheat: (1) in a balanced solution of NaCl + KCl + $CaCl_2$; (2) in a partly balanced solution of NaCl + $CaCl_2$; (3) in a pure solution of $CaCl_2$; (4) in a pure solution of NaCl (*redrawn after Osterhout*).

Ion antagonism can be easily observed on the development of roots of plants in water cultures. It has been studied in detail by Osterhout. Figure 29 shows the results of one of his experiments. In pure solutions of $CaCl_2$ and of NaCl the roots have developed very poorly. Much better growth was secured in solutions where Na was balanced by Ca, but the best development was obtained when a third cation was added, namely K. The greatest antagonism is usually observed between univalent and bivalent cations, but ions of the same valence may be antagonistic. In

general, the higher the valence of the ion, the smaller the concentration required for antagonistic effect.

Choosing different concentrations of the various ions a combination may be obtained that will suit best the development of an organism. Such an optimal combination has been termed a balanced solution. For animals, as well as for marine algae, a balanced mineral medium corresponds closely to sea water, which approaches in its composition of salts to that of the blood and lymph. This caused Loeb to say that we all carry in our bodies a part of the ocean, where once animal life was produced. A balanced medium for plants is represented by the solutions that are used for water cultures.

27. Mineral Nutrition of Plants.—In soil the mineral nutrition is much more complicated than in an artificial culture, for the plant encounters here a great variety of compounds of different elements, continually reacting with each other. Only a small part of the mineral salts found in the soil are present in water-soluble form and directly available to the plant. The largest quantities of the salts are either adsorbed by the colloidal particles, or else remain in a solid state as water-insoluble minerals or organic substances.

It has been known for some time that the water solution obtained from even the most fertile soils contains such minute quantities of nutrient salts that it cannot produce a normal development of plants. This can be proven easily by growing plants in a solution leached from a layer of soil. From this it is clear that plants must be able to feed upon adsorbed and insoluble mineral substances.

Adsorption is the mechanism whereby colloids and, in general, all finely divided substances accumulate on their surfaces substances present in a solution. The forces in operation are those of molecular attraction. Usually the phenomena of adsorption are regarded as surface reactions, caused by changes in surface tension. The union between the adsorbing and the adsorbed substances should be clearly distinguished from pure chemical reactions. Lately it has been proven that the phenomenon is more complicated than has been represented heretofore. The general idea may be accepted, however, that the adsorbed substance adheres to the surface of the adsorbing substance as a result of a decrease in the surface tension on the boundary between the solid

and liquid phases. A state of equilibrium between the two substances is thus at once established. At low concentrations of the solution, together with a large development of the adsorbing surface, the substance to be adsorbed is almost completely extracted from the solution.

In soils containing organic and inorganic colloids in abundance, the phenomenon of adsorption has an important function. It permits the explanation of many of the well-known properties of the soil, termed in general its "adsorbing capacity." This characteristic property of the soil causes the retention of dissolved substances from a solution leached through the soil, allowing only the solvent to pass. The adsorbing capacity of a soil may be very clearly demonstrated by leaching through it dyes or other organic substances. The cations and anions of various salts are rapidly adsorbed by it; as, for instance, ammonia, phosphoric acid, potassium, and magnesium. Calcium and sodium are adsorbed less; and the anions of nitric and sulphuric acid, not at all.

The adsorbed substances are very firmly retained by the soil. They cannot be leached out completely by water, but they may be displaced by other substances, especially those possessing adsorption capacity. Potassium, for instance, displaces calcium from its adsorption compounds. Thus when soil is watered by a solution of potassium sulphate, the solution leached out will contain calcium sulphate. Similarly, sodium will replace potassium, this being the reason why sometimes the addition of sodium chloride can partly substitute potassium salts in fertilizers. Substances that are easily adsorbed by soil remain largely in a bound state. Compounds of ammonium and of phosphoric acid, for example, do not go readily into solution from soil.

Experiments with plants grown on soils rich in adsorbed substances, as, for instance, those fertilized with phosphate salts and subsequently leached, have shown that plants are capable of feeding on adsorbed substances. That plants can utilize substances adsorbed on soil colloids is due to the nature of the structure of the cell walls of root hairs. They too are in a colloidal state and therefore have a considerable adsorbing capacity. As adsorption may take place only when the reacting substances are in close contact, root hairs are usually closely appressed to the soil particles (Fig. 30). Consequently, young roots possessing such hairs, when pulled out of the soil, are covered with a complete coating of soil

particles. It is almost impossible to wash these away without injuring the tissues. (Fig. 31.)

Such a close contact of the root hairs with soil particles has still another significance. The roots can utilize not only solutes and adsorbed substances, but they are able to dissolve substances that are insoluble or little soluble in water. This function of the roots was discovered by Sachs in a very simple experiment, which has since been adapted for school demonstration. A carefully polished marble plate is inserted horizontally or obliquely in the

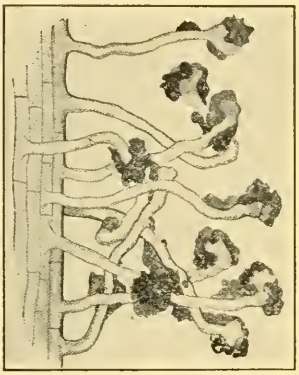


Fig. 30.—Attachment of root hairs to soil particles (*after Molisch*).

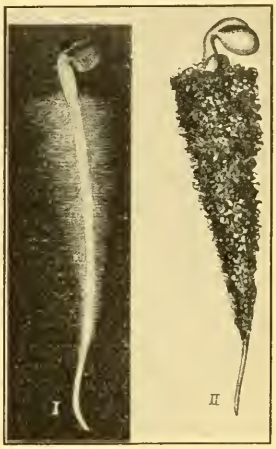


Fig. 31.—A radish seedling with a soil coating around the roots (*after Molisch*).

soil in which plants are grown, legumes being best suited for this purpose. When the roots have developed, and some of them have come in close contact with the marble plate, it is taken out and washed. On the polished surface of the plate will now be seen distinctly small traces left by the roots (Fig. 32).

It is possible to observe in the same way the dissolving effect of the roots upon natural rock phosphates. Sometimes in water-culture solutions, nutrient elements are introduced in the form of solid substances, which are gradually used by the plants during their growth, as in Crone's solution, where phosphorus is supplied in the form of water-insoluble $Ca_3(PO_4)_2$ and $Fe_3(PO_4)_2$.

The mechanism of the dissolving action of roots is not understood. An important function is performed by CO_2 excreted by

the roots. It increases the solubility of many substances in water, especially the carbonates. Some investigators (Mazè, Schulov) have found that roots excrete malic acid as well as sugar and other organic substances. In the dissolving of insoluble substances, an important part is played by the physiologically acid salts, for instance, ammonium sulphate. Roots of plants absorb more cations than anions, which causes an increase in the acidity of a medium. Prianishnikov has shown that the cereals can feed upon solid rock phosphate only in the presence of nitrogen in the form of ammonium sulphate. If fertilized with nitrates, the roots are not able to dissolve such phosphates. An important factor in dis-

FIG. 32.—Marble plate showing traces left by roots (*after Nathansohn*).

solving mineral substances in the soil is the activity of bacteria. Many of these soil organisms, for example, bacteria causing the disintegration of cellulose, among other products excrete different acids, chiefly butyric and lactic. Hence the solution of rock phosphate proceeds more easily and rapidly in humus soils rich in bacteria than in poor soils. In order to separate the activity of roots from those of bacteria, experiments must be carried out in sterile cultures. Unfortunately, there is not at present a technique which would allow the sterilization of soil without fundamentally changing all its properties; that is why we have not been able to apply sterilization methods in soil-culture experiments.

Finally, the concentration of hydrogen ions in the soil affects the availability of the insoluble substances. The experiments of Prianishnikov have shown that cereals can feed better upon rock phosphate in acid soils than in the slightly alkaline black soils. As the amount of calcium bicarbonate is one of the most important factors regulating the reactions in the soil, its content in the soil

affects the availability of the insoluble substances. It has been observed that an excess of calcium salts causes chlorosis in many plants, the iron salts having been changed to an insoluble form Such "calcium" chlorosis is especially marked in lupines and some other leguminous plants which are unable for this reason to grow on alkaline soils.

28. Means of Determining Soil Fertility. Methods of Pot and Field Experiments.—The complexity of relations accompanying the absorption of mineral nutrients from the soil does not permit one to determine exactly the degree of its fertility solely on the basis of chemical analysis. Upon a complete analysis, one will usually find even in the poorest soils such quantities of the necessary elements as will not only satisfy but will be in excess of all the needs of a plant. An analysis does not give an answer to the question as to whether these elements are present in available or unavailable form. It gives us an idea, however, of the potential amount of mineral nutrients which can be made available to the plant through the processes of weathering. An analysis furnishes most often sufficient data only on nitrogen, as this constituent of the soil is comparatively easily and rapidly transformed into substances accessible to plants. In peat-bog soils, however, even nitrogen is so firmly bound that it is unavailable to the roots of plants.

Results of a complete analysis usually give an exaggerated idea of the quantity of available mineral substances that are present. On the other hand, analysis of soil extracts or of artificially prepared water solutions does not give a correct idea of the nutritive qualities of a soil. The value thus secured usually is too small, for the roots have a higher capacity of dissolving substances than pure water.

In order to learn more exactly of its nutritional capacity, the soil is often extracted with 1 per cent hydrochloric acid, or with various concentrations of citric acid or ammonium citrate. This method is especially useful for determining the amounts of available phosphates. Data are thus secured that more closely correspond to the feeding power of the soil, but nevertheless these are only approximations. This is particularly so when it is considered that the various plants possess different nutrient requirements.

These defects in the chemical methods of soil analysis have led to a search for other means in which the plant itself would serve

as an indicator of soil fertility. The method of pot cultures is valuable in this respect. It has been worked out by investigators in agricultural experiment stations (Hellriegel and Wagner in Germany; Kossowitch, Prianishnikov and Doiarenko in Russia).

The principle of this method is the cultivation of plants on natural soils in special clay or metal pots holding from 5 to 100 kg., or even more. The environmental conditions are kept under control as far as possible. The soil moisture is held constant by means

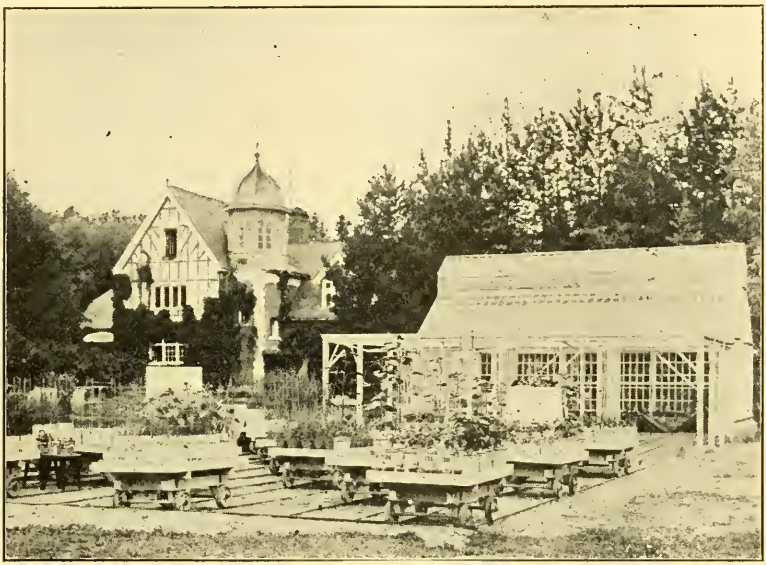


FIG. 33.—Greenhouse for pot experiments at the Institute of Applied Botany in Detskoje Selo, near Leningrad.

of daily watering of the pots to a constant weight. The pots are filled with equal amounts of carefully mixed soil and are placed on rolling platforms, which may be pushed outdoors during good weather and back into a special greenhouse during wind and rain (Fig. 33).

Soils are commonly tested in the following way: A certain number of containers, usually two to four, are fertilized with all the necessary ingredients that may be deficient in a soil, usually N, K, and P, since Ca, Mg, and S are present in almost every soil in sufficient amounts. An equal number of containers remain

unfertilized. These two series serve as controls, the first showing the maximum yield obtainable under the conditions of the experiment, the second, the minimum yield. The rest of the containers are fertilized with one of the elements mentioned. For instance, one series receives only nitrogen, another potassium, and a third,

FIG. 34.—Effects of different fertilizers on wheat in pot cultures. On the extreme left without fertilization; one next to it fertilized with nitrates; the third with potassium and phosphorus salts. The pot on the extreme right has received complete fertilization (*after Wagner*).

phosphorus. The experiment is continued throughout the vegetative period, and at the end of it the condition of plants in the different series is compared. If the addition of one of the fertilizers, for instance, nitrogen, increases the yield to its maximum, this shows that the soil is in need of nitrogen. If the same result is

obtained by adding phosphates, the soil is deficient in phosphorus. If the addition of only one element is not sufficient to secure the highest yield, then experiments are carried out with combinations of the various fertilizers, for instance, K and P, K and N, P and N. From the combinations which give the best results one can judge which of the elements are most deficient in the soil. By means of such experiments, Wagner succeeded in demonstrating very clearly

FIG. 35.—Influence of various fertilizers on peas in pot cultures. Pot on extreme left without fertilizers; one next to it fertilized with nitrates; the third with potassium and phosphorus salts. The pot on extreme right has received complete fertilization (*after Wagner*).

the capacity of leguminous plants to thrive on the nitrogen of the atmosphere. He discovered that legumes do not respond to the application of nitrates (Figs. 34 and 35).

Though the pot-experiment method is very valuable in indicating which of the nutrient substances is present in the soil and which fertilizers must be added, it cannot furnish correct quantitative data, for in pots the roots of plants are confined to a volume of soil considerably smaller than when grown under field conditions.

This is the reason why the requirements for fertilizers by plants are always higher in pots than in the field. For exact determination of the amounts of fertilizer to be added, it is more expedient, therefore, to use the field method. An experimental field is usually divided into plots of some definite size and to each is applied either one or a combination of fertilizers. By determining separately the yield from each plot, it is easy to find what the fertilizer requirements are. This procedure of determining the needs of a soil is widely used by the experiment stations and in experimental field work. Expressing it figuratively, Timiriazeff says that in applying this method we ask the plant directly what is deficient in a given soil for its welfare.

A still more direct answer to the question of how much and what substances the plant may receive from the particular soil is given by Neubauer's method which has been widely applied in Germany during recent years and is beginning to find its way into other countries. The procedure of this method is as follows: a great number (up to 100) of seedlings are grown for 2 to 3 weeks in a flat glass cup on a small amount (100 g.) of soil. The plants are then separated from the soil, dried, and carefully analyzed. It is supposed that the plants have had time to absorb all of the available mineral substances from the soil and that the quantity of the mineral elements, particularly phosphorus and potassium, revealed in the analysis, after deducting the amount present in the seeds, corresponds to the amounts of these elements in the soil, which are available to the plants. Neubauer's method is no doubt very ingenious, but evidently it cannot be applied to all soils. Moreover, the very small amounts of the substances that have to be determined call for very sensitive and very exact methods of chemical analysis. Then, too, in making calculations from 100 g. and applying them to acre plots, every error of the analysis is multiplied many thousand times. At present, this method is not very generally applied.

29. Yields as Determined by the Amount of Nutritive Substances. Law of the Minimum. The Action of Growth Factors. Before the application of artificial fertilizers it is necessary to learn which of the elements are needed for a certain soil. In contrast to the natural fertilizers, such as manure or the various organic products, by the term "artificial fertilizers" are understood the different mineral compounds obtained in part

from the soil itself, as for example, Chile saltpeter, rock phosphates and Stassfurt potash, or secured as byproducts of various industries. Ammonium sulphate, for instance, is obtained in the process of purification of illuminating gas, and Thomas slag is formed during the smelting of ore containing phosphorus. Some artificial fertilizers are the products of direct synthesis, such as Norwegian saltpeter or synthetic ammonia. Natural fertilizers contain all the substances necessary for the plant, while artificial fertilizers are unbalanced, usually furnishing only one or two of the needed elements. It is clear, therefore, that the application of a potash fertilizer to a soil unfertile as a consequence of phosphorus deficiency, is a useless expense and will not lead to an increased yield. Naturally, the transition from a primitive type of agriculture, based principally on fertilization with manure, to more intensive forms, in which the application of artificial fertilizers becomes common, requires the development of a system of experiment stations, where the requirements of the local soils can be studied, as such experiments are beyond the capacity of individual farmers.

The application of artificial fertilizers is subject to the law of the minimum, which was established by Liebig at the time of the introduction of artificial fertilizers into agricultural practice. According to this law, as worked out by Hellriegel, the yield is determined by the amount of that necessary element which is present in a minimum quantity in proportion to the demands of the plant. By gradually increasing the content of this element, the yield will be increased in proportion to the amount applied until another element will begin to act as a limiting factor. One of Hellriegel's experiments showed that with the addition of 28 mg. of nitrogen the yield was 2.99 g.; with 56 mg., 5.70 g.; with 112 mg., 10.80 g.; with 224 mg., 21.07 g.; or that by doubling the amount of nitrogen used, the yield also was doubled.

The law of the minimum has been applied not only to the influence of ash elements on yield, but to all other factors which determine the development of plants, such as light, water, temperature, etc. In this modified usage, this law has served as a foundation for Blackman's law of limiting factors, which was mentioned in Art. 14.

Recently the law of the minimum has been seriously criticized by Mitscherlich and his collaborators. He points out that a proportionality between the amount of some nutrient element and

the yield may be observed only within comparatively narrow limits and that with an increase of the amount of a given element, the effect of increasing applications of this element decreases and finally ceases to affect the yield. This dependence of yield on a certain limiting element cannot be expressed graphically by a straight line, as in the case of a simple proportionality, but by a logarithmic curve (Fig. 36). Mathematically, Mitscherlich expresses this relationship by the formula

$$\frac{dy}{dx} = (A - y)K$$

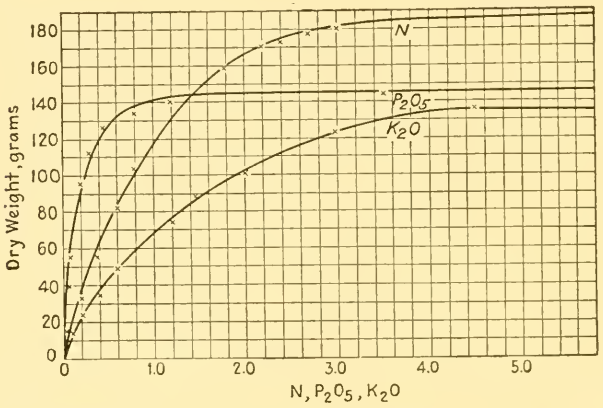


FIG. 36.—The influence of increasing doses of fertilizer on the yield, according to Mitscherlich.

where A is the maximum yield obtainable under given conditions, y the yield actually obtained, x the amount of fertilizer used, and K a constant. By integrating and transforming, the following formula, convenient for calculations, is obtained:

$$\log (A - y) = \log (A - Y_0) - Kx,$$

where Y_0 is the magnitude of yield on unfertilized soil. Knowing this magnitude, as well as the amount of substance applied and the yield obtained after its application, it is possible to calculate the constant K. According to Mitscherlich, this constant is wholly independent of the plant, being determined exclusively by the factor of yield obtained. This formula shows that when definite amounts of a fertilizer are gradually supplied, the yield increases

proportionally to the difference between the yield actually obtained and the maximum yield, consequently, the increase gradually diminishes with its approach to the maximum yield. This dependence of the yield on increasing amounts of fertilizers Mitscherlich terms not the law of minimum, but the law of action of growth factors.

When several factors are acting simultaneously, that is, when a number of them are increasing at the same time, Mitscherlich has noted also significant deviations from the law of the minimum. He found that the increase in one of the nutrient elements slightly

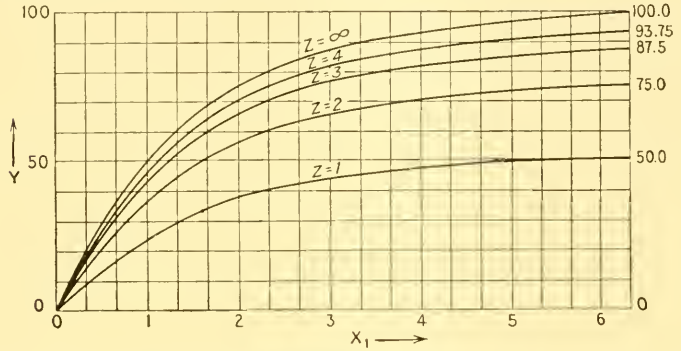


FIG. 37.—The influence of the simultaneous change of factors X and Z on the yield y, according to Mitscherlich.

augments the effect of the other nutrient substances. Thus in the case of nitrogen deficiency, a potash fertilizer may increase the yield, though, according to the law of minimum, this should not be the case. The simultaneous action of two factors is graphically represented in Fig. 37.

The formula proposed by Mitscherlich has met with many objections and has been amended by other investigators. Consequently, it must be regarded only as a general scheme, one requiring further analysis. Russel, for instance, has observed that when very small quantities of mineral substances are added to the soil, their action is considerably weaker than it should be according to Mitscherlich's law. He explained this as a direct consequence of soil adsorption. Hence the greatest effect is obtained neither from too small nor too large, but from average doses of fertilizers. In this connection, especially sharp criticisms have

been called forth by the statement of Mitscherlich that the constants of the action of the growth factors are perfectly equal in all plants. The difference in the requirements of different plants with regard to mineral substances having been repeatedly and incontestably established, this statement seems improbable.

30. The Use of Mineral Substances by Various Plants. Absorption of Soil Nutrients at Different Stages of Growth.—The chemical composition of the ash of plants is variable, depending largely on the environmental conditions under which they are grown, their general properties, and their age. We have already noted that the dryer the climate and the greater the amount of salts in the soil, the higher will be the ash content of a plant. One cannot conclude from this, however, that the accumulation of ash in the plant is merely a mechanical process—simply the result of evaporation of water from the leaves, the salts dissolved in it remaining in the plant. Plants growing close together on the same soil always show differences in ash composition, thus indicating clearly the selective accumulation of some elements in preference to others. One of the reasons for such a selective accumulation of mineral substances in the cell is the unequal permeability of the absorbing root cells: they are readily permeable to water, but the substances dissolved in the water enter with greater difficulty (see Art. 37). Besides, the different ions penetrate with different facility into the cells, and from there pass into the other parts of the plant. The other possible reasons for unequal accumulation of different substances in the cells will be discussed later. Similarly in various parts of the plant the composition of the ash differs considerably. The following table contains data on the composition of ash of the vegetative organs and seeds of some important agricultural plants. As is customary, the percentages are calculated not on the basis of pure elements but as their oxides, and the amount of each oxide is given as the percentage of the total weight of pure, carbon-dioxide free ash.

The table shows that seeds are rich in phosphorus and potassium, elements very necessary for the building of new organs and very often lacking in the soil. The stems and leaves of cereals often show a high silicon content, and high calcium content in flax and clover. In the subterranean storage organs, large amounts of potassium are present. This seems to suggest an important rôle of this element in the storage of carbohydrates.

THE COMPOSITION OF ASH OF SOME AGRICULTURAL PLANTS

	K_2O	Na_2O	CaO	MgO	Fe_2O_3	P_2O_5	SO_2	SiO_2	Cl
I. Seeds:									
Wheat	30.2	0.6	3.5	13.2	0.6	47.9	.....	0.7	
Corn	29.8	1.1	2.2	15.5	0.8	45.6	0.8	2.1	0.9
Clover	35.3	0.9	6.4	12.9	1.7	37.9	2.4	1.3	1.2
Flax	26.7	2.2	9.6	15.8	1.1	42.5	.....	0.9	
Beans	41.5	1.1	5.0	7.1	0.5	38.9	3.4	0.6	1.8
II. Stems and leaves:									
Wheat	13.6	1.4	5.8	2.5	0.6	4.8	.....	67.5	
Corn	27.2	0.8	5.7	11.4	0.8	9.1	.....	40.2	
Clover	27.2	0.8	29.3	8.3	0.6	10.7	.....	6.2	
Flax	34.1	4.4	24.8	15.0	3.7	6.2	.....	6.7	
Buckwheat	46.6	2.2	18.4	3.6	.....	11.2	.....	5.5	
III. Tubers and roots:									
Potatoes	60.0	3.0	2.6	4.9	1.1	16.9	6.5	2.1	3.4
Sugar beets	53.1	8.9	6.1	7.9	1.1	12.2	4.2	2.3	4.8
Turnips	45.4	9.8	10.6	3.7	0.8	12.7	.....	1.8	5.0

Because of the varying ash content and composition and the different capacity of plants to feed upon slightly soluble soil compounds, they naturally possess different requirements for soil nutrients and require, therefore, different fertilizers. Potatoes, for instance, need potash, and cereals need phosphorus. Moreover, in agricultural practice, various amounts of ash elements are removed with the harvest, and, therefore, various crops exhaust the soil to a different degree. The average quantities of ash elements extracted from the soil by certain agricultural plants are given in the following table, the figures indicating the number of kilograms per hectare.

	K_2O	CaO	MgO	P_2O_5	SO_3	SiO_2
Winter cereals	50	14	9	23	5	106
Spring cereals	50	18	10	20	6	86
Legumes	60	60	16	27	10	10
Potatoes	106	35	20	33	16	8
Beets	184	40	27	32	12	6

The table shows that the highly cultivated crops, like potatoes and legumes, exhaust the soil more than do the cereals and thus

have a higher fertilizer requirement. Legumes, therefore, cannot be regarded as crops that enrich the soil. Though increasing its nitrogen content, they exhaust the mineral reserve.

In economical practice, where almost all the mineral elements taken from the soil are returned to it in the form of manure and other refuse, soil exhaustion takes place slowly. But with the development of modern marketing, agricultural products are not used locally, but are sold mostly in the city, and the mineral substances contained in the crop are lost forever. Therefore, the farmer must return to the soil the substances removed. This statement was clearly formulated for the first time by Liebig and termed by him "the law of return." He took this law literally, stating that without exception all the mineral elements removed from the soil must be returned, preferably in the form of artificial fertilizers, which he was the first to introduce into agricultural practice. In his first artificial fertilizer mixtures he had all the ash elements, including Mg, Na, S, and Si.

It was soon found, however, that the law of return must not be taken literally, that it is quite unnecessary to return some of the elements, for inexhaustible reserves of them are contained in the gradually disintegrating rocks. That is why at the present time usually phosphorus, potash, and nitrogen are returned to the soil without paying any attention to the other elements.

Plants do not absorb the elements at the same rate during the different stages of growth. As a rule, most of the annuals absorb the greater part of the salts necessary for their development before the flowering stage. In some plants as, for instance, corn, all the soil nutrients required for their further development are already accumulated in the stem at the time of flowering when their further absorption ceases almost completely. Not all of the individual elements found in the ash are absorbed at the same rate during the development of the plants. Potash, for example, accumulates before phosphorus. This may be due to the observed fact that potash takes part in the metabolism of carbohydrates, which proceeds more energetically during the vegetative period, while phosphorus is more important for successful flowering and fruiting.

The composition of ash of the various organs of the plant changes also with age. Leaves of the beech, picked in May, were found to contain per 100 parts of ash, 42 parts of K_2O, 32 parts of

P_2O_5, 14 parts of CaO, and only 1.6 parts of SiO_2. Those collected in June showed already a markedly altered composition, namely, 17 parts of K_2O, 8 parts of P_2O_5, 42 parts of CaO, and 21 parts of SiO_2; and when gathered in October, before abscission, K_2O made up 7 parts; phosphorus, 5; calcium, 51; and silicon, 30. The less necessary elements seem to accumulate with age, while the amount of potassium and phosphorus shows a relative reduction.

This comparative decrease of potassium and phosphorus, however, does not indicate an absolute decrease of these elements. It may result from a relative increase in the total quantity of other elements. Analysis in which not the general amount of ash but a definite number of leaves, for instance 1,000, served as the unit of calculation, have proved that the amount of all the elements gradually increases during the vegetative period. Shortly before leaf fall a return movement may be observed in the leaves of the more mobile elements, like K and Mg. The greatest part of the ash content remains in these organs. It may again become available to plants after the leaves have dropped and decayed in the soil.

31. Plants with Special Nutrient Requirements.—Most plants are capable of growing on different soils, for almost all contain sufficient nutrient substances for their development. Hence, climate permitting, it is possible to raise the same crop plants in widely differing soil zones. Still not all soils can satisfy the requirements of different plants to the same degree, and the distribution of the wild flora can often serve as an excellent indicator of soil properties. A few characteristic plants may be considered as definite indicators of certain soil conditions, not only of its chemical composition but its physical character and water-holding capacity as well.

One of the most characteristic examples of such adaptation is shown by plants growing in saline soils, called "halophytes." They are usually found on seashores, where the soil is heavily impregnated with salt water, or in salt marshes and the alkali soils common in regions of dry climate, where weathering proceeds more rapidly than leaching, and soluble salts accumulate. Plants of the usual type do not grow or grow poorly on such soil. They are replaced by halophytes, which are distinguished by considerable anatomical and physiological peculiarities. These plants often possess fleshy stems and leaves, thereby resembling those of the type of cacti (Fig. 38). This characteristic structure is hereditary,

for it is reproduced by the plants when grown on soils where no salts are present. But with an increase of the salt concentration in the soil, succulence increases, which after all may be regarded, in a measure at least, as one of the direct results of high soil salinity.

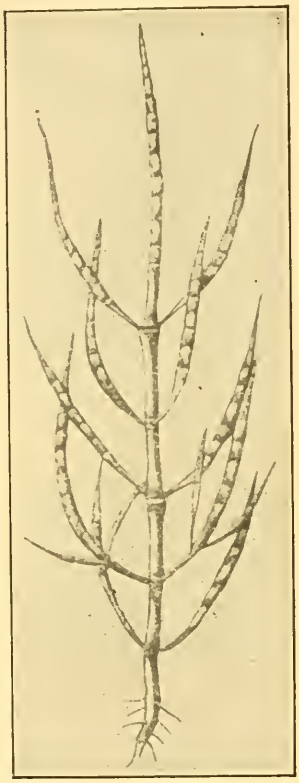


FIG. 38.—*Salicornia*, a fleshy plant from saline soil (*after Schimper*).

Because of the abundance of sodium chloride in some saline soils, large quantities of this salt are found also in the ash of plants native to such soils. Some of the saline plants, as for instance *Statice gmelini*, can remove the surplus through special glands, however, and consequently have a much lower salt content. Sodium chloride is not an absolute physiological necessity for saline plants. They can grow on soils which are not saline. The experiments by Keller, though, have shown that the addition of this salt improves their development (Fig. 39). This is one of the reasons why they are able to displace from alkali soils all the other plants, most of which are injured by certain quantities of salt. Excessive amounts of sodium chloride, however, can destroy even the most tolerant of saline plants.

There are plants which accumulate considerable amounts of nitrates in a manner similar to the halophytes. These plants thrive best on soils rich in nitrates. They are often found near dwellings where the soil contains much refuse. Among such plants are included several species of *Atriplex* and *Chenopodium*, the nettles, sunflower, and many others. It is interesting to note that the concentration of nitrates in the sap of such plants is many times higher than the concentration of these salts in the soil solution. Hence it is legitimate to call these plants nitrate accumulators, as sea algae may be termed accumulators of iodine and bromine.

In their relationship to the calcium content of the soil plants may be divided in two great groups; those which like lime, or calciophiles, and those that avoid lime, calciophobes. These two groups are linked by a series of intermediate plants. They may be sharply separated only by considering their extreme representatives. These differences, however, are of a purely physiological character. They cannot be distinguished by any anatomical or morphological peculiarities.

FIG. 39.—*Salicornia* plants growing without sodium chloride, on the left; and with the addition of sodium chloride, on the right (*after Keller*).

Not many of the higher plants are definitely calciophobes. Good examples of such plants are the chestnut (*Castanea vesca*), sphagnum moss, and several other plants of sphagnum bogs.

As calcium represents one of the indispensable elements for the development of higher plants, it seems at first sight incomprehensible how there can be plants which do not grow on calcareous soils. Yet the ash of such lime-avoiding plants contains normal amounts of CaO. This becomes understandable, however, if it is remembered that an increased or decreased lime content results in chemical and physical changes of the soil. Soils that contain much lime have a neutral or slightly alkaline reaction advan-

tageous for bacterial activity and the accumulation of humus, which is favorable for plant growth, while in acid soils fungi develop and an acid humus accumulates which is injurious for many plants. It is interesting to note that in water cultures all plants, calciphilous as well as calciphobous, require the addition of a sufficient amount of calcium ions for their normal development. With sufficient acidification of the solution, even the most calciphobous plants support perfectly well a high concentration of calcium salts. On the contrary, with alkalization even a small surplus of calcium is injurious. Thus the relation of plants to lime is closely connected with their relation to the concentration of hydrogen ions in the soil. The physical properties of a soil, its porosity and its heat absorption, are improved by an increase in the lime content.

32. Effects of Hydrogen-ion Concentration of the Soil.—Of all the cations affecting the development of plants, H^+ ions are the most active. One of the principal properties of acids and alkalies, as well as of water, is the faculty of dissociating into cations and anions. In water, as well as in any water solution of acid or alkali, therefore a certain number of free ions of H^+ and OH^- is present and the so-called neutral reaction indicates not the complete absence of hydrogen ions, but the equal concentration of hydrogen and the hydroxyl ons. Water is a neutral medium. Water dissociates into H^+ and OH^- ions according to the law of mass action.

$$\frac{H^+ \times OH^-}{H_2O} = K.$$

Since the concentration of undissociated water molecules, H_2O, in water and in weak aqueous solutions, is much higher than the concentration of H^+ and OH^- ions, H_2O may be regarded as constant in water, as well as in aqueous solutions of acids and alkalies. Then the product of the concentrations of H^+ and OH^- will be equal to the constant $H^+ \times OH^- = Kw$. The determinations of different investigators have shown that the dissociation constant for water is: $Kw = 10^{-14.14}$ gram-molecules per liter. In a neutral solution, the concentrations of hydrogen and hydroxyl ions are equal; consequently, the concentration of $H^+ = 10^{-7.07}$. As H^+ and OH^- ions are present in both acid and alkaline solutions, only in different proportion, the magnitude produced by them being constant, not only the acidity but also the alkalinity may be expressed by indicating

the concentration of the H^+ ions. The active acidity of a solution is usually indicated not in gram-equivalents per liter or normality, but by the negative logarithm of the normal concentration denoted by the sign *p*H, the hydrogen-ion exponent. Thus, the concentration of the H^+ ions at neutral reaction is denoted by *p*H = 7. As *p*H is a negative logarithm, the lower the designation of *p*H, the higher the concentration of the H^+ ions; and the more acid is the reaction, and vice versa, the higher the *p*H is, the more alkaline is the reaction.

The development of plants is in general possible only within comparatively narrow limits of concentration of the H^+ ions.

The *p*H value of the soil may vary from 3 to 11. The first figure indicates a very acid soil, the second a strongly alkaline one that may only be attained by the accumulation of magnesium salts. Calcium salts can increase the *p*H value of the soil only to 8.4. Chernozem [1] soils, rich in carbonates, have also an alkaline reaction, podsol [2] soils, on the contrary, are acid. Soils of bogs are very acid.[3]

Recent investigations have shown that the hydrogen-ion concentration is one of the essential factors in determining plant distribution. Some plants grow best at one *p*H concentration, others at another. Very often it is possible to judge more or less exactly the active acidity of the soil from the specific complex of a meadow vegetation. The Danish investigator, Olsen, has proven that the predominance of *Deschampsia flexuosa* among the meadow grasses

[1] Chernozem (black earth).—A very productive soil developed under subhumid or semiarid climatic conditions on open grassland. The soil is characterized by a very dark surface horizon which varies in depth from 1 to 4 ft. and which possesses a well-developed granular structure. The soil does not contain soluble toxic salts, but both the organic and mineral colloids are saturated with calcium and magnesium. The normal reaction is neutral and the typical topography smooth. The largest continuous area of chernozem lies in the southern half of western Europe (Russian steppe) and extends eastward into Siberia.

[2] Podsol (ash-like soil).—A soil developed under forests of moderately humid regions. It is characterized by an ashy-gray or nearly white horizon just below the leaf litter. The horizon varies in thickness from several inches to more than 2 ft. Below the ashy-gray or podsolized horizon lies a very heavy, compact dark-brown or reddish-brown horizon of varying thickness, usually 1 to 2 ft. The podsolized horizon is typically acid in reaction. The topography varies from undulating to rough. Typical podsols are to be found in the forested portion of northern United States.

[3] Bogs in northwestern United States are frequently alkaline.

indicates a *p*H of 3.5 to 3.9; of *Carex goudenougii*, a *p*H of 4.5 to 4.9; *Deschampsia caespitosa*, a *p*H of 5.5 to 6.4; and *Tussilago Farfarus*, a *p*H of 7.5 to 7.9. These figures have been confirmed by artificial cultures of these plants in nutrient solutions. It has been shown that each plant has its optimum, maximum, and minimum concentration of *p*H for growth (Fig. 40).

The numerous investigations of the last few years, especially the work of Arrhenius, have helped to elucidate the response of plants to the reaction of the soil. It has been ascertained that different species show different requirements with regard to the

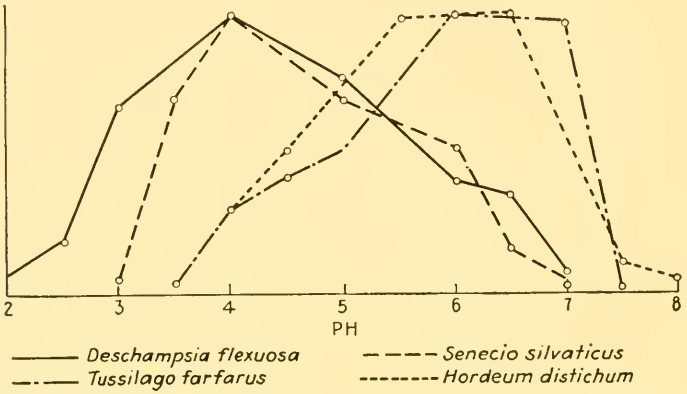


FIG. 40.—Growth of several plants in solutions of different hydrogen-ion concentration (*after Olsen*).

optimum concentration of H^+ ion and are moreover distinguished by a different degree of resistance to changes in this connection. While oats, rye, turnips, and potatoes yield the highest returns with a *p*H of about 5 to 6; wheat, barley, beet root, and alfalfa thrive better with neutral or slightly alkaline reaction. Not only different species, but even different races of the same plant show different requirements with regard to *p*H.

The physiological influence of the actual acidity in soils is still very poorly explained and great divergence exists in the views which are held among different investigators. It has been established that the *p*H exerts a great influence on the penetration of salts into the cell; the increase of acidity promotes the absorption of anions, while the increase of alkalinity, on the contrary, promotes the absorption of cations. On the other hand, there is a

hypothesis that at optimum *p*H for a given plant, the permeability of the protoplasm to salts is the least, and that with the shifting of *p*H to the one or to the other side of this optimum the permeability may increase too much, resulting in injury to the plant.

The soil, which is the natural medium for the development of plants, represents a much more complex substratum than the solutions used in water and sand cultures. According to many authors, the influence of *p*H of the soil may be directly upon the plant or may act indirectly by affecting the solubility of the nutrients and poisonous soil substances. Thus, it is known that acid soils, or physiologically acid fertilizers, applied to the soils promote the solubility and absorption of phosphates. The solubility of the Fe and Al salts equally depends on the *p*H of the soil. The investigations of Uspensky with algae have shown that the more iron the organism requires, the more the *p*H optimum for its development shifts to the acid side, since the solubility of iron increases with acidity. On the contrary, algae which are susceptible to an excess of iron, for instance, *Cladophora*, are injured in consequence of a high Fe content, in water, the reaction of which is lower than *p*H = 7. The chlorosis of some plants on alkaline soils rich in calcium carbonate may be explained, not so much by the injurious influence of the reaction as such, as by the lack of Fe insoluble in an alkaline medium in the presence of calcium and phosphates. But chlorosis is observed in the soil less frequently than in water cultures, since the soil is usually rich in organic substances producing complex compounds with Fe. These compounds are retained in the solution even with an alkaline reaction.

On the other hand, the *p*H of the soil may influence the plant by acting on the solubility of the poisonous Al^{+++} ion. Aluminum is quite insoluble at acidities from *p*H of 4.5 to 8.5. In a more alkaline or more acid medium, the solubility of aluminum sharply increases. Plants may suffer greatly in acid and in strongly alkaline soils not only from the H and OH ions, but also from the toxicity of aluminum. At the present time, these questions are being investigated in detail by botanists as well as by soil scientists, but they are still far from being solved.

Besides the actual acidity determined either electrometrically or by indicators described in physical chemistry, the titrable acidity determined by the usual methods of titration, likewise may have an influence on plants. Solutions with the same actual

acidity may show different amounts of titrable acidity, dependent on the degree of dissociation of the acids in the solution and their buffer action. "Buffer action" is the property of the solution to minimize changes of H^+ concentration on dilution or on the addition of H^+ or OH^- ions. Buffer action depends on the presence of mixtures of weak acids in the solution, or of bases, with their salts. Well-buffered solutions may be mixtures of carbonates, of alkalies, and alkaline earth metals with carbon dioxide, or mixtures of phosphates of different basicity in which there are potential reserves of anions and cations able to neutralize the H^+ or OH^- ions. The soil always contains, to a greater or less degree, the above-mentioned combinations. For this reason, a soil solution is much more stable in regard to *p*H than aqueous solutions. The soils showing the greatest buffer action are those rich in organic substances, for instance peaty or muck soils. Soils showing the least buffer action are sandy soils or gray, leached soils, and "podsols."

Besides the actual acidity, soils possess also potential acidities apparent only when the soil is cultivated with the application of mineral fertilizers. This form of acidity is explained by the adsorption of the added salts with the liberation of free acid or by the replacement of the base of the salt by aluminum. The newly formed salt is hydrolyzed in turn into the slightly dissociated aluminum hydroxide and the strongly dissociated free acid, which determines the concentration of hydrogen ions. The significance of these types of acidity for plants has not been fully studied.

Part II

WATER RELATIONS OF THE PLANT

CHAPTER IV

IMPORTANCE OF WATER IN THE LIFE OF THE CELL. THE CELL AS A COLLOIDAL OSMOTIC SYSTEM

33. Saturation of the Cell with Water as an Essential Condition of Life. The Water Content in Different Parts of the Plant.—Organic life originated in water, and the most primitive organisms such as amoebae and algae are able to live and to develop only in a water medium. The migration from water to dry land, which finally transformed the algae into higher flowering plants, entailed a series of complex adjustments in the structure and reproduction of plants. The principal physiological properties of living matter, however, have remained the same. The cell of the land plant requires for its normal function almost the same degree of saturation with water as the cell of the submerged plant.

Of all the substances surrounding the plant, water occupies a prominent position. It is the most important ingredient of the body of a plant, constituting 90 per cent or more of the green weight. Yet, in a strict sense, water is not an organic compound and therefore not a permanent constituent of the cell. It continually changes, being utilized in the process of evaporation, and then again being replenished from the soil. In other words, water is a part of the environment, permeating the whole organism, and it is in water that all the complex chemical and physical processes take place, the sum total of which is called life.

The amount of water in the different parts of the plant fluctuates within rather wide limits. In organs consisting chiefly of active cells, such as the leaves and the young tips of roots and stems, the water content approaches 90 to 95 per cent. In the wood, where the majority of cells are made up of dead, more or less thickened walls, the amount of water is but 50 per cent, while in such dormant organs as buds and seeds the water content is still lower. Thus, in wintering buds in which feeble vital processes are still going on, 40 to 50 per cent of water may be found. In per-

fectly dormant seeds showing no signs of life, the water content is but 10 to 15 per cent. Seeds give an excellent illustration of how much the vital processes in plants depend on a definite degree of saturation. Perfectly viable seeds containing only hygroscopic water may remain unaltered during many months, and even years, without showing any signs of life. As soon as they are soaked in water, they at once begin to germinate. If seeds obtain less water than is required for their complete saturation, but more than they usually contain when air dried, for instance 20 to 25 per cent, respiration, the elimination of carbon dioxide and absorption of oxygen is initiated. This as well as a number of other processes may eventually lead to the loss of power of germination. Under these conditions, no growth is secured. This incomplete awakening of the seeds by partial moistening is of great importance. It shows that various vital processes require different degrees of saturation with water. The highest amount is necessary for growth and other processes connected with constructive activities, the lowest, for respiration and other processes of degradation.

One of the reasons why water is so essential for the vital functions in a plant is the fact that many chemical reactions may be effected only in a water solution. Protoplasm itself, the principal substrate of all vital processes, must be saturated with water in order to be active. Protoplasm is often defined as a semiliquid substance of mucilaginous character. Such substances have been termed colloids, which means gluelike, or substances in the colloidal condition. It has been established in recent years that the same substances may be obtained in crystalloidal as well as in colloidal state.

34. General Conception of the Colloidal State. Fundamental Properties of Colloids. Phenomenon of Swelling.—The colloidal condition of a substance, in terms of physical chemistry, is a state of very high dispersion, while the medium in which the minutely divided particles are suspended is called the dispersion medium.

Three types of dispersed systems may be distinguished: (1) coarsely dispersed systems with particles of the dispersed phase not less than 0.1μ (micron = thousandth of a millimeter); (2) colloidal systems in which the particles of the dispersed phase range from 0.1μ to 0.001μ; and (3) molecular, or ionic, dispersed systems in which the division in the dispersed phase is so great that it is represented by separate molecules or ions that have lost all connec-

tion with one another. The coarsely dispersed systems are mechanical mixtures whose heterogeneity may be readily observed under the microscope. Such systems are usually unstable. Their dispersed phase is either precipitated or rises to the surface on standing. With sufficient subdivision of particles, however, a greater stability is obtained. As examples of such stable mixtures are fine suspensions of clay, which do not settle for many days, or common India ink, a suspension of minute carbon particles in water. Sometimes, the suspended particles are so small that they remain imperceptible under a common microscope. In such cases one has to resort to a so-called ultra-microscope, under which the object is examined not with transmitted light, but in the dark field of vision with very strong lateral illumination. The solid particles reflect the rays of light and become visible as bright dots or circles against the dark field of vision, showing the heterogeneity of the medium studied. The reflection of light rays from the sun or from a projecting lantern makes visible the minute dust particles floating in the air, which are invisible in transmitted or weakly diffused light.

Similar to suspensions are emulsions, or mixtures of two immiscible fluids, such as, for example, water and oil. Ordinary milk is such an emulsion, in which particles of fat swimming in water may be seen under the microscope. Artificial emulsions are readily obtained by shaking vigorously two fluids that as a rule do not mix, or by diluting with water an alcohol solution of a substance that is insoluble in water. In this manner, Perrin prepared the emulsion of mastic on which he carried out his famous investigations on Brownian movements. The dispersion of the substance attains its maximum in a true solution, in the solvent of which are distributed separate molecules and even ions of the dissolved substance. At this degree of dispersion the stability of the system is very high, and the dissolved substance may be separated from the solvent only with great difficulty. Such separations can be obtained only by means of freezing, or by evaporation of the solution. The resistance to separation is expressed by the lowering of the freezing point or the raising of the boiling point of the solution.

Colloidal systems occupy an intermediate position between coarse mixtures and true solutions. The degree of dissociation in colloids is considerably greater than in coarse mixtures, and the

dispersed particles are much smaller, usually not exceeding 0.1μ. Accordingly, they are more stable than coarse mixtures. The particles of colloidal mixtures, called micellae, since the time of Naegeli, are, however, considerably larger than molecules. They represent solid or liquid aggregates which, due to their small dimensions, possess in aggregate an immense surface, separating them from the dispersion medium. Consequently, in colloidal systems an important rôle is played by surface phenomena, especially by adsorption.

It is this enormous increase of the surface which determines the greater part of the specific properties of colloidal systems. How rapidly the surface increases with an augmented degree of dispersion and diminished size of the separate particles may be seen, for instance, from the well-known calculations of Wolfgang Ostwald. If we take a cube with sides of 1 cm., its surface will be 6 sq. cm. If this cube is divided into smaller cubes with sides of 1 mm., their number will be 10^3, with a total surface of 60 sq. cm. In diminishing the sides to 0.1μ, the maximum size of colloidal particles, we obtain from the initial cube 10^{15} small cubes with a total surface of 60 sq. m. If the sides of the cube are diminished to 0.001μ, the minimum size of colloidal particles, the number of small cubes will reach 10^{21}, with a total surface of 6,000 sq. m. If these calculations are applied to the very fine animal charcoal, which is used in the sugar industry for decolorizing sugar solutions, we shall see that 1 cu. m. of charcoal with grains 1μ in diameter gives a total adsorbing surface of 6 sq. km. It might be thought that in true solutions with a still higher degree of dispersion and still smaller dimensions of the particles, molecules, or ions, the total sum of the surfaces ought to be greater than in colloidal systems, and that correspondingly the processes connected with the increase of surface area ought to be more marked. This, however, is not observed, since with such a degree of dispersion every ion and every molecule is bound to water particles, which form as it were a shell around them. Consequently, the dispersed systems containing molecules and ions are without an active surface. The maximum manifestation of surface forces is connected not with maximum dispersion, but with a certain intermediate degree of it, which characterizes the colloidal systems.

Depending on whether the micellae are liquid or solid aggregates, two types of colloidal substances are distinguished, emul-

soids and suspensoids. A large number of organic colloids belong to the first group; the majority of inorganic colloids belong to the second. Water solutions of emulsoids are very stable. When dried, they absorb water with great force, and thus may be readily dissolved again. They are called, therefore, hydrophilous or lyophilic colloids. Once having been dried, solutions of suspensoids, on the contrary, cannot be restored to the original state.

Not being molecular but micellar dispersed systems, solutions of colloidal substances differ considerably from true solutions. They have been called, therefore, pseudo-solutions, or sols. A most conspicuous difference is their relation to highly porous plant or animal membranes, such as bladder or parchment paper.

Colloidal sols are not able to pass through membranes, as their large micellae are retained in the pores, while true solutions pass readily. Upon this difference depends the well-known method of dialysis, by which colloids may be separated from crystalloids. A vessel covered with a bladder membrane or with parchment paper is filled with the liquid to be dialyzed and is then immersed in another vessel in which the water is continually renewed. The crystalloids diffuse through the bladder and are carried away by the water current. The colloidal solution remains in the dialyzer (Fig. 41).

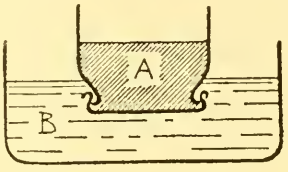


FIG. 41.—A dialyzer.

Another difference between crystalloids and colloids lies in their property of lowering the freezing point of the solvent. In true solutions this lowering is very marked. Thus with water solutions of non-electrolytes it may be as high as 1.85° C. for every gram-molecule per liter of solvent. In colloidal solutions, where the particles of the dispersion phase are large and their number relatively small, the lowering of the freezing point is usually so insignificant that it cannot be conveniently measured.

Separated from the solvent, the molecules of the dissolved substance usually form structures of regular crystalline nature, wherefore these substances have been called crystalloids. Not so with colloids. Separated from water, their micellae turn into amorphous bodies termed "gels." They always retain more or less water or any other substance that served as the dispersion medium for the colloid. The structure of gels is to a certain degree the inverse

of that of sols. The original dispersed substance builds a continuous mass in which the remains of the former dispersion medium appear as the dispersed phase.

Sometimes the transformation of a sol into a gel is effected without the removal of the greater part of the dispersion medium. Thus, for instance, the congealing of gelatine takes place with the cooling down of the sol to ordinary temperature. Such gels saturated with water are called "jellies." There is reason to suppose that the colloidal substance of such jellies forms something of the nature of a complex network, in whose meshes the water is retained in an immobile condition.

When dried, jellies decrease markedly in volume, losing their water content. But only the first portions of water are given up as readily as in colloidal solutions. Further loss of water meets with progressively increasing resistance. The last portions of water may be removed only by heating to 100° C. On the other hand, when a dried jelly is immersed in water, it imbibes the latter, and increases its volume rapidly. This phenomenon is termed swelling of the colloid. The first portions of water are imbibed by the dried colloid with a force as high as 1,000 atmospheres. The weight that the swelling colloid is able to lift may serve as a measure of this force. Especially convenient for such experiments are dry seeds, which in swelling develop a pressure great enough to burst a bottle. When such seeds are placed in an empty skull, their pressure causes the bones to separate at the sutures.

As the water content of the gel increases, its water-absorbing power gradually decreases, finally becoming zero. In this condition the colloid ceases to attract water. This is a state of perfect saturation. Some colloids preserve in this condition the character of solid though jelly-like, bodies, and therefore are sharply distinguished from the surrounding water. Such colloids of limited swelling capacity are, for instance, cellulose and starch. To this group also belongs protoplasm. Other colloids begin to lose the attraction between their particles when perfectly saturated with water, or even before this stage is reached, and are gradually converted into a solution. Such colloids of unlimited swelling are, for example, the protein substances and the gums as gum arabic. There is no sharp line of demarcation between colloids of limited and colloids of unlimited swelling. The swelling of gelatine, for instance, is limited at low temperatures and unlimited at

high. Starch, when heated, becomes pasty, turning into a colloid of unlimited swelling capacity.

The phenomenon of swelling and the reciprocal phenomenon of desiccation play an important rôle in the life of the cell. The majority of vital processes can proceed only in a protoplasm sufficiently saturated with water.

35. The Cell as a Colloidal System. Osmotic Properties of Cell Membranes. General Laws of Osmosis.—The plant cell consists of two principal parts, the cell wall and the protoplasm, each of which may be regarded as a colloidal system. The cell wall, is made up largely of cellulose—a gel of limited swelling capacity and of a highly porous structure. The protoplasm within the wall is a sol, though very dense, still exhibiting the characteristics of a liquid body. This is shown by a number of properties. Protoplasm possesses a marked surface tension. When escaping from the wall, for instance, when zoospores are formed by algae, it assumes a spherical shape, typical of drops of liquid. The frequently observed movement of protoplasm within the cell also points to its liquid nature. As protoplasm does not mix with water, it belongs to colloids of limited swelling.

Living protoplasm is always saturated with water. Its limited swelling capacity, as well as the characteristic permeability for substances dissolved in water, make us suppose, however, that the water in it is not a continuous but a dispersed phase. The dispersion medium is probably a mixture of protein and fatlike substances. This question, however, has not yet been solved completely.

In very young cells, the whole cavity is filled with a solid mass of protoplasm. In time, vacuoles containing a watery sap arise in this mass. These vacuoles finally merge, forming one central vacuole. The typical vegetative cell, therefore, may be considered as a double-walled sac filled with cell sap. The cellulose membrane of the cell is the outer wall of this sac, the layer of semiliquid protoplasm, forms a lining within it (Fig. 42).

These two membranes, separating the cell contents from the surrounding medium, are endowed with very different properties. The wall is a stiff porous gel. Owing to its solidity, it easily preserves its shape and shows but a limited capacity of distention. Being a liquid, though very dense, the protoplasmic sac shows an almost unlimited distention capacity. Not being able to maintain

a definite shape, it readily fills all hollows and projections of the outer wall.

The properties of the walls delimiting the cell determine not only the form of it, but also the laws governing the penetration of substances from the surrounding medium into the cell. These laws, in their turn, determine the conditions of absorption of nutrient substances by the cell. The presence of a rigid wall around every cell prevents the absorption of solid food. It is well known that one of the fundamental physiological differences between the animal and the plant organism is that the animal cells, amoebae, and infusoria, do not have a rigid wall, and consequently are able to absorb and digest particles of solid food, while plant cells depend exclusively on substances dissolved in water.

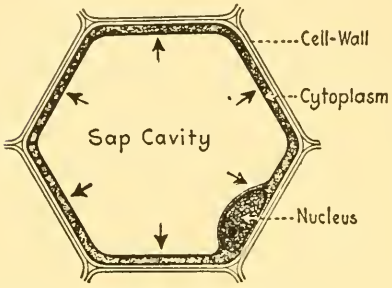


FIG. 42.—A diagram of an adult plant cell. Arrows show the direction of osmotic pressure (*after Sinnott*).

Substances dissolved in water are frequently conceived of as being in a state approaching that of the gases. As has been shown they are molecularly dispersed. Their separate molecules, or even ions, are scattered among the molecules of the solvent. They have lost the power of cohesion and are in a condition of continual motion, similar to that of gas particles. Though this movement is considerably slower in a liquid than in the gaseous state, the physiological property is the same. The particles of the dissolved substance tend to be uniformly distributed in the available space; the volume of the solvent. In other words, dissolved substances exhibit the phenomenon of diffusion. The slowness of diffusion of such substances may be due to the rather high density of the medium in which they move as well as to the fact that each of their molecules or ions is bound to the nearest water particles, which form around them a shell of water. Otherwise, the laws governing the diffusion of gases may be applied to diffusion of liquids in the cell. The fundamental law that the rate of diffusion is in inverse proportion to the size of the particles is likewise applicable here. The lower the molecular weight of a substance, the more rapidly it will spread through the solvent. The large particles

of a substance of colloidal character move much slower than the molecules of crystalloids.

If a diffusing substance meets a membrane in its path, diffusion becomes more or less complicated. If this membrane is porous and the pores are filled with the same solvent, then there is less complication. In this case, however, the rate of diffusion is still more reduced. The difference between the speed of the moving substance and the size of its particles increases. Only the largest colloidal aggregates are retained by the membrane. Membranes of this type are used in dialysis, by means of which colloids may be separated from crystalloids. The cellulose of the cell wall is a membrane of this character. It is permeable to almost all substances dissolved in water.

A sac made of a membrane, such as animal bladder, parchment paper, or a hardened film of collodion, is filled with a solution of cane sugar and tightly closed. If placed in water, this sac will swell rapidly, its walls will become turgid, and finally it may rupture when it is unable to withstand the pressure from within. If instead of closely tying up the opening, a glass tube is inserted into it, we shall soon see that under the influence of internal pressure the level of the liquid in the tube begins to rise, first rapidly, then slower and slower, until it stops at a certain definite level, after which it begins to fall again.

Following the changes in the contents of the sac, it is found that at first a rapid entrance of the water takes place, which leads to a considerable increase in volume. Afterwards, the diffusion of the dissolved substance from the sac into the surrounding water becomes more and more obvious, and finally the concentration of the outer and of the inner solutions becomes almost equal, and the walls begin to collapse. The entrance of the water into the sac is called "endosmosis," the outward escape of the solute, "exosmosis." The whole apparatus, the sac with the inserted tube, has been called an osmometer by Dutrochet, the French botanist, who was the first (1826) to study this phenomenon. The diffusion of liquid and dissolved substances through a membrane has been given the general name, *osmosis*, while the hydrostatic pressure, developing as a result of an increase of endosmosis over exosmosis and swelling of the sac has been called "osmotic pressure."

Further investigations have shown that besides minutely por-

ous membranes which only slightly check the diffusion of water and its solutes, there exist membranes which allow water or the solvent to pass, but which are wholly impermeable to the solutes. Such membranes, first discovered by Traube, in 1867, have been called semipermeable or selectively permeable ones. The semipermeable membrane which has been most studied is the precipitation membrane of colloidal copper ferrocyanide, arising at the place of contact of a copper sulphate solution with a potassium ferrocyanide solution. The penetration of substances through such membranes takes place not by diffusion through the pores of the membrane, but by solution of the substances in the membrane. Only such penetration in which the primary rôle is played not by the size of the particles of the moving substance, but by its solubility in the membrane, is real osmosis. In *Dutrochet's* osmometer only diffusion takes place, slightly modified by the friction of the particles against the walls of the pores. The protoplasmic lining of the cell is a semipermeable membrane. The study of such membranes is of great importance in obtaining information on the processes that take place in the cell. This study was first undertaken by Pfeffer in his classical "Osmotic Investigations" (1877). His work started a new epoch in the study of the properties not only of semipermeable membranes but also of water solutions in general. Pfeffer showed that these membranes readily let through water but are quite impermeable to the substances dissolved in it, and that no exosmosis takes place through them. Therefore, the pressures developed by solutions contained within such membranes are not temporary but permanent, and attain considerable magnitudes. Thus, a 2 per cent solution of cane sugar produces an osmotic pressure equal to 102 cm. of mercury above atmospheric pressure, while in Dutrochet's osmometer even with very concentrated solutions, a pressure of 10 to 15 cm. is obtained with difficulty.

The pressures developed by weak solutions are quite considerable. The precipitation membranes of copper ferrocyanide, on the other hand, are very delicate and easily ruptured. Hence, in measuring these pressures Pfeffer proceeded in the following way: he took a porous porcelain cylinder such as is used in batteries, filled it with a solution of copper sulphate, and submerged it in a solution of potassium ferrocyanide. A membrane was precipitated in the small pores of the porcelain, the walls of these pores

providing a sufficiently strong support for the membranes to sustain a pressure of several atmospheres (Fig. 44).

Pfeffer's osmometer shows a certain similarity to the plant cell. In the latter, we also find a readily permeable, yet solid support in the form of the cell wall and the adjoining layer of semipermeable protoplasm. Therefore, this osmometer has frequently been called an artificial cell. In comparison with a real

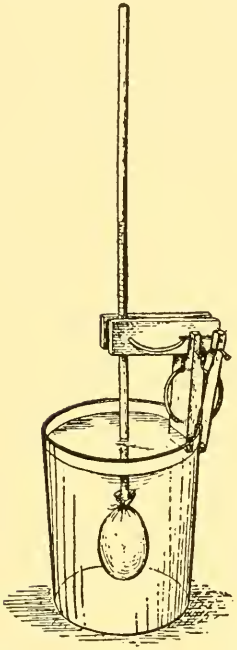


Fig. 43.—Osmometer (*after Dutrochet*).

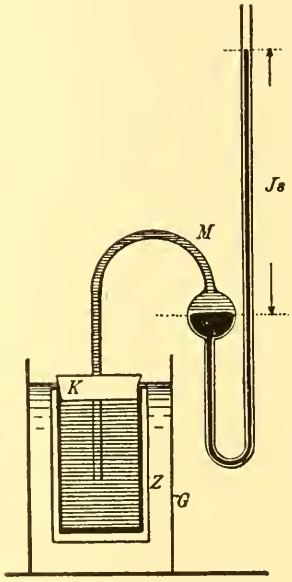


Fig. 44.—Pfeffer's osmometer (*after Molisch*).

cell capable of being distended, Pfeffer's osmometer has rather rigid walls and naturally it is unable to alter in volume.

Pfeffer established that the osmotic pressure increases in direct proportion to the concentration of the solution and to the absolute temperature. It is governed by the laws of Boyle and Gay-Lussac, established for gaseous pressures. Even the absolute magnitude of gaseous and osmotic pressures, at a concentration of one gram molecule of substance per liter, has proved to be the same, namely, 22.4 atmospheres. In spite of the unity of the laws governing

osmotic and gaseous pressures, these two pressures show certain differences, the chief of which is that gases always develop a pressure on the walls of the container enclosing them, while the osmotic pressure of solutions shows itself only in case they are placed in a container with a semi-permeable membrane separating the solution from water. Osmotic pressure arises only when the solution and the solvent are separated by a semipermeable membrane and, according to the determination of Findley, it is equivalent to the hydrostatic pressure produced.

The osmotic pressure produced when an osmometer is immersed in water, is explained by the attraction between the molecules of the solvent and those of the solute, determined by the influence of the chemical affinity between them. Water enters the osmometer under the influence of this affinity until the hydrostatic pressure on the walls of the osmometer becomes equal to the osmotic pressure of the solute. If the hydrostatic pressure acts upon a mercury column, as in Pfeffer's osmometer the rise ceases when the pressure of the mercury column balances the tendency of the solution to increase its volume by the absorption of water. The height of the mercury column indicates the magnitude of osmotic pressure which may be determined as the equivalent of the external pressure to which the solution must be subjected to prevent the penetration of the solvent through the membrane, *i.e.*, to stop the increase in the volume of the solution.

36. Impermeability of Protoplasm and the Phenomenon of Plasmolysis. The Osmotic Properties of Cell Sap and Methods of Its Determination.—The easiest way of convincing oneself that protoplasm is but slightly permeable, or is impermeable, to the substances dissolved in water, is by observing cells with colored cell sap; for instance, the cells of the red beet root or the leaves of red cabbage. While alive, these cells may be kept in water for many days without losing their pigment. But, as soon as the structure of the protoplasmic membrane is destroyed, for instance, by boiling or even by heating at 60 to 70° C., or by the addition of some poison, the colored sap diffuses into the surrounding water through the cell walls. More exact analytical methods have shown that other substances dissolved in water, as for example, the salts and sugars, are not lost to any extent from the living cells. Otherwise, the very existence of submerged plants would be impossible.

The phenomenon of impermeability of the protoplasm to sub-

stances dissolved in water may be detected also in another way. If a cell, preferably with colored cell sap, is placed into a sufficiently strong solution of some harmless substance, such as sugar or potassium nitrate, the following results may be observed under the microscope. First, the cell decreases somewhat in volume, after which the protoplasm begins to retreat from the cell wall. If the solution is very strong, the protoplasm will shrivel up into a ball-like mass containing the highly concentrated cell sap (Fig. 45). The space between the cell wall and the protoplasmic sac will be filled with the external solution, which has penetrated through the wall. This phenomenon of shrinkage of the protoplasm has been called "plasmolysis."

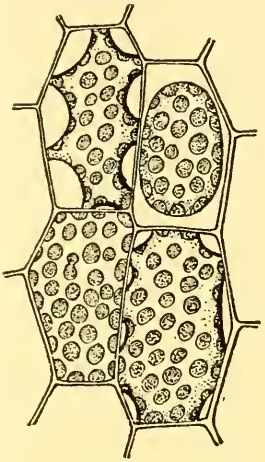


FIG. 45.—Successive stages of plosmolysis in cells of a moss leaf (*after Molisch*).

The different stages obtained in watching the cell in water or in a solution may be explained in the following way: The protoplasm is readily permeable to water and impermeable to substances dissolved in the cell sap. When the cell is surrounded by pure water, these substances attract the latter, causing the protoplasmic sac to increase its volume. As a consequence of this increase in size, the protoplasm distends and becomes appressed to the cell wall, transmitting its pressure to the latter (Fig. 42). This condition is obtained when the elastic tube of a bicycle tire transmits the pressure of the air pumped into it to the more solid outer rim. As a result of this pressure, the wall of the cell is in a rigid condition known as "turgidity," and the volume of the cell is slightly increased.

If a cell is put into a concentrated solution of any substance, then, owing to the impermeability of protoplasm, this substance will exert an osmotic pressure on the latter. The direction of this pressure, however, will not be toward the outside of the cell, but inward. The cell wall being permeable, the solution readily passes through it. Under the influence of the pressure, water escapes from the cell sap into the outer solution and the plasma sac decreases in volume. If the total molecular concentration of the

external solution is higher than the total molecular concentration of the cell sap, the pressure of the external solution directed inward exceeds the pressure of the cell sap directed outward. Consequently, turgor pressure disappears, and the cell decreases in volume. Moreover, the protoplasmic sac withdraws somewhat from the cell wall, which, with lessened internal pressure, contracts slightly.

The phenomenon of plasmolysis is of importance in many ways. In the first place, it shows a difference in osmotic properties of the protoplasm and the cell wall. Second, it may be used frequently to indicate whether the cell under investigation is alive or not, because impermeability is peculiar only to living cells. Finally, plasmolysis enables one to determine with great accuracy the magnitude of the osmotic pressure within every living cell.

A method for determining the osmotic pressure in cells was devised by De Vries as early as 1884. It is based on the following considerations: The higher the osmotic pressure of the external solution above the osmotic pressure of the cell sap, the greater is the shrinkage of the protoplasm. On the other hand, the smaller the decrease in volume of the protoplasmic sac, the less it will withdraw from the cell wall, thus showing a smaller difference between the two pressures. If a concentration of the outer solution is found which causes but an incipient shrinking of the protoplasm, which is usually observed in some corner of the cell, one then may assume that this concentration of the outer solution balances the concentration of the cell sap within. Since the concentration of the external solution is known, it is easy to calculate its osmotic pressure and from this the almost equal osmotic pressure of the cell sap.

The problem then is to find a concentration of the external solution that produces only incipient plasmolysis. For this purpose a series of solutions of increasing concentration is prepared, differing from one another by the same magnitude, for instance, by 0.1 mol. Thin sections of the plant organ under investigation are placed in these solutions. The highest concentrations induce strong plasmolysis; the weakest, no plasmolysis whatever. Somewhere in the middle, a concentration will be found which will induce only the incipient stages of plasmolysis. The concentration of this solution corresponds to the molecular concentration of the cell sap. Hence it has been called the osmotic value.

This plasmolytic method of determining the concentration of the cell sap is simple and expedient and therefore widely used. It is especially valuable when one has to deal with single cells or with tissues of small volume, from which it is difficult, or even impossible, to obtain the amount of sap required for the usual physicochemical methods of determination of the concentration of solutions. When the osmotic pressure is to be ascertained in sufficiently massive organs yielding much sap, then, instead of the plasmolytic method, the cryoscopic method may be used. The sap is squeezed from the minced or frozen tissues by means of a strong press, and the freezing point is determined. Knowing that a gram-molecular solution of any non-electrolyte lowers the freezing point of water by 1.86°, it is easy to calculate the molar concentration of the sap from the freezing-point depression. Both methods give the same results, thus confirming the correctness of the values obtained by plasmolysis.

The absolute magnitude of osmotic pressure in the cells is rather high. In land plants it most frequently ranges from 5 to 10 atmospheres, in submerged fresh-water plants, it is considerably lower—from 1 to 3 atmospheres. In plants native to saline soils or dry habitats the osmotic pressure is very high, reaching 60 to 80, or even 100 or more atmospheres. The magnitude of pressure depends chiefly on the accumulation in the cell sap of soluble substances of small molecular weight. In the majority of halophytes with an extraordinarily high pressure the substances are most frequently either sodium chloride or other mineral salts. In some plants, osmotic pressure is induced chiefly by sugars and organic acids.

Such immense magnitudes of the osmotic pressure in the cells appear improbable at first sight and give rise to questions as to how the thin cell walls are able to withstand the tension. A more detailed study of the question shows, however, that the magnitudes of the osmotic pressure, calculated according to the concentration of the cell sap, considerably exceed the actual hydrostatic pressure on the cell walls. The solution absorbed from saline soils, surrounding the cells of halophytes, shows a very high concentration, and the cell wall is subjected to the excess of the inner over the outer pressure. If these plants are placed into pure water, the abrupt increase of hydrostatic pressure of the contents leads to a strong distention of the cell walls and sometimes to their

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rupture. Rupture rarely takes place, however, as with the distention of the cell its volume rapidly increases, and simultaneously the concentration of the cell sap falls.

Another instance of cells with very high concentration of the cell sap is the pollen of many plants. According to Art. 35, the pollen, as long as it is dry, shows no osmotic pressure. When transferred to the stigma, it finds there a sugary liquid of rather high concentration, balancing the internal concentration of the pollen cells which in germinating increase their volume gradually. In pure water, the pollen frequently bursts, owing to the excess of inner pressure. This explains the harmful influence of rain and fog on the setting of seed. From this, the various modes of protecting the pollen against the influence of atmospheric precipitation, realized by very intricate arrangements in the structure of the flower, become comprehensible.

The osmotic pressure in the cell is not constant. It varies continually, depending on the chemical processes of the cell. Thus, when starch is hydrolyzed into sugar, the pressure is increased considerably. It is decreased as a result of the reverse process, the accumulation of starch at the expense of sugar. The imperfect oxidation of sugar, accompanied by an accumulation of organic acids of small molecular weight, as for example, oxalic acid, also leads to an increase of osmotic pressure. By changing the chemistry of its internal processes, the plant is able thereby to regulate to a certain degree its osmotic pressure.

The plasmolytic method, in which the pressure is calculated from the concentration of the plasmolyzing solution, is most frequently used for determination of the osmotic pressure. Very often only the concentration isosmotic (*i.e.*, causing an equal pressure) with the cell sap is recorded. This magnitude has been termed the "osmotic value." Following the example of DeVries, we formerly used solutions of potassium nitrate for this purpose. Now, cane sugar is preferred, as it is harmless to the protoplasm, while strong salt solutions frequently induce pathological changes. Besides, for solutions of cane sugar there now exists carefully calculated tables showing the osmotic pressure for any concentration, and translated into terms of atmospheric pressure.

37. Substances Causing Temporary Plasmolysis. Permeability of the Protoplasmic Membrane to Different Substances and the Problem of Its Composition and Structure.—DeVries,

who first investigated the phenomenon of plasmolysis, noted that some substances, for instance, salt and sugar, induce persistent plasmolysis lasting for hours; while others, as glycerin and urea, cause only a temporary plasmolysis. In a few hours the shrunken protoplasm regains its former shape and once more becomes closely appressed to the cell wall. De Vries explains this phenomenon quite correctly. The substances slowly penetrate into the cell, their concentration outside and inside the cell becoming equal, and the distending influence of the substances contained in the cell sap again manifesting itself.

Further experiments have established that the number of substances that enter readily into the cell is rather considerable. Many of them pass into the cell so easily and rapidly that no plasmolysis is induced. To this category belong chiefly the alcohols, the ethers, and a series of narcotics, such as chloroform, etc. A careful investigation of various substances in respect to their capacity to penetrate into the cell has drawn Overton's attention to the fact that the protoplasm is readily permeable to those substances which are easily soluble in fats. Those insoluble in fats penetrate with greater difficulty, or not at all. And since the osmotic diffusion of a substance through a septum is conditioned, in the first place, by its solubility in the material forming the septum, Overton draws the conclusion that the protoplasmic membrane must be composed chiefly of fats or fatlike compounds, such as lecithin and other lipoids.

It must be noted that, according to the view held by Pfeffer and now shared by the majority of investigators, the properties of the protoplasmic sac are not uniformly the same throughout its extent. The external layer, the one nearest to the cell wall, and the internal layer lining the vacuole, show impermeability to a considerably higher degree than the intermediate layers. Pfeffer has called these layers the plasma membrane or " Plasmahaut."

In Overton's opinion, this surface layer consists chiefly of fatlike substances which accumulate there, owing to the fact that fats decrease surface tension. And according to a fundamental law of physical chemistry, substances decreasing surface tension are bound to accumulate near the surface (Gibb's law). The lipoid theory of Overton, therefore, explains well the ready permeability of the cell to narcotics, alcohols, and esters. But it supplies no answer to the question why water, though insoluble in

fat, rapidly enters the cell, and why salts and sugars, which are readily soluble in water, penetrate but very slowly or not at all. Hence, this theory must be regarded only as a proximate idea, requiring elaboration and correction.

The question of the permeability of the protoplasm to different substances is, on the whole, rather complicated, and still far from a definite solution. A large number of substances absolutely necessary for cell nutrition, such as sugars and mineral salts, move freely from cell to cell in the body of a living plant. The same substances, however, induce persistent plasmolysis lasting for hours, and, consequently, under these conditions do not penetrate into the cell. This contradiction leads to the supposition that in plasmolysis the properties of the protoplasmic membrane have changed in the direction of an increased impermeability. The recent experiments of Sabinin are especially illustrative in this regard. This investigator has shown that by immersing the roots of seedlings in very weak solutions of different salts, the same salts will be found in the fluid extruded from the cut surfaces of the above ground parts of the plant under the influence of exudation pressure. These experiments have shown that the root cells at least are much more permeable to salts than one might think on the basis of plasmolytic experiments.

These results, as well as the ready permeability of protoplasm to water, lead to the supposition that the structure of the protoplasmic membrane is made up not only of lipoids but also of protein substances, which swell easily in water. Lepeschkin conceives of the protoplasmic membrane as an unstable chemical compound of protein and lipoids. Other authors conclude that these substances do not combine chemically, but form a sort of complex emulsion. It is interesting to note that the permeability of the protoplasm to substances dissolved in water is not a constant phenomenon. It varies considerably, according to changes in external and internal conditions. Thus, for instance, narcotics and light increase the permeability of the protoplasm, while strong solutions, as has been shown, considerably decrease it. By means of certain irritations, the permeability of protoplasm may be greatly augmented. Thus, in case of extremely sensitive plants, as for instance, *Mimosa*, these changes lead to sharp movements which are perceptible to the eye. This variation of permeability perhaps is connected with the variations in the relative amounts of

protein and lipoid particles in the surface layer. With the accumulation of the first, permeability increases, with the predominance of the latter, it decreases.

38. Conditions of Accumulation of Soluble Substances in the Cell. Importance of Chemical Alteration in Substances for Their Accumulation. Donnan's Membrane Equilibrium.—According to the laws of diffusion, such substances as salts and sugars, which are soluble in water and are absorbed by the cell, must continue to enter the cell until the concentration of each substance becomes equal, both outside and inside the cell. As the plant usually obtains rather dilute solutions of nutritive substances, it is evident that their entrance into and accumulation within the cell require special conditions.

A most important prerequisite is the chemical change of the absorbed substances. For instance, when carbohydrates are stored in the tubers of the potato, the sugar obtained by the cells from the leaves is transformed directly into starch, which is insoluble in water. The concentration of sugar in the cells of the growing tuber is, therefore, extremely low and does not impede the diffusion of new amounts of sugar. The same is observed in ripening oil-bearing seeds. The only difference is that in this case fats are accumulated at the expense of the soluble carbohydrates. Protein compounds are formed from amino acids, and so on. As a general rule, the substances entering the cell are subject to chemical transformation which assures their uninterrupted absorption.

To demonstrate the dependence between the accumulation of substances in the cell and their transformation, one may use a sac of hardened collodion, representing a model of the plasma sac lining the plant cell from the inside. Water diffuses readily through the collodion membrane, salts penetrate with greater difficulty, while colloidal substances hardly pass at all. If such a sac is filled with water and immersed in the solution of some salt, for instance in ferric chloride (Fig. 46), the salt will penetrate into the sac until the outer and inner concentrations become equal. No accumulation of iron within the sac will take place. If instead of pure water the sac is filled with a solution of tannin which produces with iron a dark-colored compound of colloidal nature, the solution will remain inside the sac and will bind the iron ions penetrating into the sac. As a result, the concentration of the iron ions inside the sac will be always considerably lower than on the outside,

and finally the sac will absorb all of the iron from the outer solution.

As protoplasm is rather permeable to substances dissolved in water, they might be washed out from the cells. This leaching, however, is prevented due to the fact that the reserve substances are either insoluble in water, as starch and oil, or are in a colloidal state, as protein, and inulin. When seeds germinate or when organs containing food reserves are emptied, these substances return to their former soluble and mobile condition. They leave the cells in which they had accumulated, moving towards the growing organs.

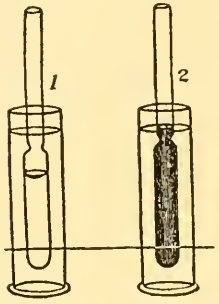

FIG. 46.—Colloidal sac with tannin placed in solution of an iron salt.

This general mechanism of the absorption and accumulation of substances in the cell, however, does not always hold true. There are cases when soluble substances accumulate in great quantity, and remain in the same state in which they were when entering the cell. Thus, for instance, in the bulb of the common onion are stored considerable amounts of glucose. In pigweed and other plants much potassium nitrate accumulates. It must be noted that the concentration of nitrates in the root cells exceeds their concentration in the soil solution. Attempts have been made to explain this occurrence by the fact that sugar and potassium nitrates are present in these cells in special unstable combinations with some substances of the cell sap. All efforts of isolating such combinations have failed, however.

At the present time, the explanation of these phenomena is sought in the so-called membrane equilibrium of Donnan.

If a membranous sac impermeable to colloids containing a readily ionizable salt, one of whose ions is of a colloidal character, such as sodium proteinate, a sodium salt in which the rôle of an acid is played by some protein, is immersed in water, the sodium ions, though able to pass through the membrane, will not diffuse out of the sac, being retained by the electrostatic attraction of anions and cations. If some easily penetrating salt is added to the water, for instance, sodium chloride, then in the process of osmosis the Na and Cl ions will diffuse through the septum and finally there will be established an equilibrium in which products of the

concentrations of the anions and the cations of the readily diffusing salt must be equal on both sides of the membrane. Schematically, this may be represented as follows: in the initial state, the following concentrations may be on both sides of the membrane, denoted by a vertical line:

Na^+	$Proteinate^-$	Na^+	Cl^-
C_1	C_1	C_2	C_2

Then, in the state of equilibrium, if the anions of protein cannot pass through the membrane, while the other ions pass readily, we shall have

Na^+	$Proteinate^-$		Na^+	Cl^-
$C_1 + X$	C_1	X	$C_2 - X$	$C_2 - X$

According to Donnan, at equilibrium the products of the concentrations of the diffusible ions must be equal on both sides of the septum. Then:

$$(C_1 + X)X = (C_2 - X)(C_2 - X).$$

From this equation may be calculated the relation between the ions of sodium and of chlorine on both sides of the septum. This calculation shows that the higher the concentration of the sodium proteinate in comparison to the concentration of sodium chloride the less complete will be the equalization of the concentrations of NaCl in the surrounding solution and in the osmometer, and the more it is retained in the surrounding liquid. Let us give some figures illustrating this "membrane equilibrium" of Donnan.

Initial Concentrations		Final Concentrations, NaCl	
Na proteinate	Na Cl	Inner solution	Outer solution
0.01	1	0.497	0.503
0.1	1	0.476	0.524
1	1	0.33	0.66
10	1	0.083	0.917
100	1	0.01	0.99

These figures show that the large amount of sodium proteinate makes the membrane appear as if impermeable to NaCl and this salt ceases to penetrate into the sac of the osmometer.

If a salt with another cation than one connected with the proteinate within the osmometer is taken, for instance KCl instead of NaCl, another correlation will be obtained. The cation of this electrolyte introduced into the surrounding solution, in this case K, will be attracted towards the interior of the membrane by the anion of protein. The anion, on the contrary, will be forced outwards. Donnan illustrates this correlation by the following table:

Initial Concentration		Final Concentrations					
		K		Na		Cl	
Na proteinate	KCl	Inside	Outside	Inside	Outside	Inside	Outside
0.1	1	0.5	0.5	0.5	0.05	0.5	0.5
1	1	0.66	0.33	0.66	0.33	0.33	0.66
10	1	0.90	0.10	9.2	0.8	0.10	0.90
100	1	0.99	0.01	99.0	1.0	0.01	0.99

With a great abundance of colloidal ions within the osmometer, if compared to the concentration of salts in the surrounding liquid, for instance 100 : 1 in the last line of our table, the almost complete disappearance of K (99 per cent of the original amount) from the surrounding medium and its accumulation within the osmometer may be observed. At the same time, the almost complete "expulsion" of the Cl ion from the osmometer is observed, in spite of the fact that both ions pass readily through the membrane and enter into no chemical reactions within the osmometer.

In an analogous way may be explained the accumulation of different kinds of ions in the plant cells containing a considerable amount of protein substances which show the properties of ampholytes, that is, substances which act as weak acids and weak bases and, therefore, are able to induce the accumulation of cations, as well as of anions. As the composition of the colloidal substances in the cells changes continually, quantitatively as well as qualitatively, however, the conditions of Donnan's equilibria prove very complicated in them, and are still far from being sufficiently understood. The protoplasm being impermeable not only to colloids but also to many electrolytes, for instance to organic acids,

these combinations too may determine the establishment of Donnan's equilibria between the cell and the surrounding medium and may promote the accumulation in the plant of many anions and cations in much higher concentrations than are found in the surrounding medium; for instance, in the soil solution. At the present time, attempts are being made to reconstruct the whole theory as to the entrance of substances from the soil into roots on the basis of the conditions discovered by Donnan.

39. Changes in the Colloidal State of Protoplasm and Its Influence on Osmotic Properties. Coagulation and Death of Protoplasm.—The property of not being permeable to substances dissolved in the cell sap is preserved by protoplasm only as long as it is alive. As soon as protoplasm dies or is killed, it loses its impermeability, and the substances dissolved in the cell sap diffuse out. The cooking of food is based to a considerable extent on the property of killed cells releasing the substances contained in them. This refers especially to fruits and vegetables which in a raw state are less digestible, as a considerable number of their cells, remaining unbroken during mastication, pass through the alimentary canal without yielding up the enclosed nutritive substances.

The fixation of the protoplasm for subsequent staining of the enclosed nucleus, chondriosomes, etc., for cytological study is based on the loss of permeability by the cells. The living protoplasm is impermeable to almost all stains except a few used for staining in the living condition: neutral red, methylene blue. To differentiate and to stain the component parts of a cell, it is necessary to kill it by a poison such as alcohol, acetic and osmic acids, etc., which will not disturb the minute structure of all these formations.

The loss of impermeability by the killed cell results from changes in the state of the colloids of the protoplasm. From the condition of a sol they are transformed to that of a gel, the degree of their dispersion decreases, and their micellae form irregular aggregates between which canals, readily accessible to water and solutions, are opened. This phenomenon is called "coagulation." It may be easily illustrated by heating the white of an egg, which is somewhat diluted with water.

The coagulation of protoplasm may be brought about by different causes, such as high temperature, poisons, salts of heavy

metal, acids, alkalies, as well as by an excessive withdrawal of water or even by mechanical pressure.

In all cases, coagulation leads to death of the protoplasm. This shows that a comparatively high degree of dispersion of the colloids making up protoplasm is closely connected with its vital properties.

The phenomenon of coagulation is peculiar not only to protoplasm and albuminous substances in general, but also to other colloids of emulsoid and of suspensoid character. With suspensoids is obtained almost always an irreversible coagulation. In many emulsoids, on the contrary, the precipitated or dried colloid may return to the condition of a gel. This kind of coagulation is called reversible. The same colloids may show in some cases a reversible, in other cases, an irreversible coagulation. Thus, the white of an egg coagulates to an irreversible state when heated or when treated with poisons. When dried or subjected to the influence of strong solutions of neutral salts, it enters the condition of a gel, which may be dissolved again.

In many instances, protoplasm may be dried to a state of complete air desiccation when usually 10 to 12 per cent of so-called hygroscopic moisture remains without losing its vitality, *i.e.*, the faculty of returning to the sol condition. Thus, seeds which in ripening lose all free water and become air dry, simultaneously suspend all vital processes. When moistened, however, they once more return to activity. The same is true of many cryptogams, such as mosses, lichens, and some ferns. Not only their organs of reproduction, but the whole thallus of these plants may be reduced to an air-dry condition. They return to activity when moistened. In such a dried protoplasm, however, some processes must be going on which alter the dispersion of its particles. When stored for a very long period, dry seeds lose their power of germination. Under similar conditions, dried cryptogams lose their capacity of returning to life. Similar coagulation is observed when photographic plates are kept for a long time. Their emulsion gradually becomes more and more coarse grained and the plates lose their sensitivity to light.

40. Influence of Low Temperatures on Plants. Freezing and Frost Killing.—Changes in the condition of the colloidal substances of the protoplasm supply a clue to the knowledge of the complex phenomena taking place in the plant at temperatures below freez-

ing. Some plants, such as the cucumber, tomato, dahlia, and potato, are easily killed by even a mild frost. Others are extremely hardy, as, for instance, winter rye and the conifers. Frost-killed plants look as if scalded; they lose their turgidity, and their leaves rapidly turn brown and become dry. When such fleshy tissues as the potato tuber or the beet root thaw out after having been frozen, water flows out of them as easily as from a sponge.

This ready loss of sap by frost-killed organs was explained for a long time as being due to the fact that the freezing water, in expanding, ruptures the cell walls. This supposition, however, proved false. Microscopical observations have shown that water contained in the cell walls is frozen first and that ice is formed not within the cell but in the intercellular spaces (Fig. 47). The cell walls in frost-killed plants remain uninjured. The cause of the death of these plants must be sought, not in the rupture of the cell walls, but in the changes of the protoplasmic membrane, primarily its coagulation. This coagulation is the result of the formation of ice in the intercellular spaces and the withdrawing of water from the cells. The cell sap becomes increasingly more concentrated, while the protoplasm is gradually deprived of water. Besides, the protoplasm becomes exposed to the pressure of the growing ice crystals. As a result, an irreversible coagulation of the colloidal substances of the protoplasm takes place. After thawing, it is dead and has lost its impermeability.

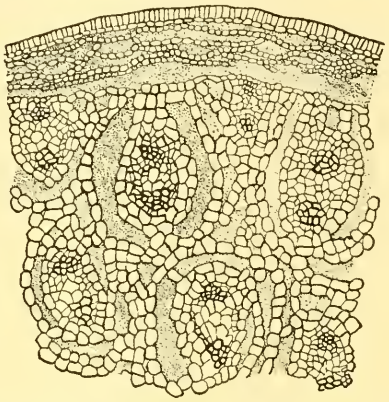

Fig. 47.—Accumulation of ice in the intercellular spaces of the frozen stem of *Fritillaria* (*after Sachs*).

Hence, frost killing must be regarded not as the direct influence of cold on the protoplasm but as an indirect influence, the desiccation of protoplasm due to the freezing out of water. One of the most direct proofs of this statement is the fact that in the state of undercooling, without ice formation, the plant is able to endure such low temperatures as would kill it if freezing had occurred.

Not all plants succumb with equal ease to the formation of ice. Some of them, the most sensitive ones, are killed in the very beginning of this process. Others, such as the cabbage or the onion, are able to stand moderate freezing. Those in a third group may freeze to a quite hard condition and thaw out afterwards without losing their vitality. Many plants of the polar regions have this capacity. Some winter cereals are able to stand 15 to 20° C. even in snowless winters. Still more cold resistant are the dormant buds of deciduous trees and the needles of conifers.

The reason for this hardiness was sought first in the fact that because of the small amount of water in these dormant organs, especially the buds and needles, no ice could form. This supposition, however, has proved erroneous. Observations have shown that even these organs freeze at 15 to 20° C. With still lower temperatures they become brittle as glass. Moreover, all such external adaptations as the layer of cork covering the branches, the bud scales, etc., which, for a long time, were regarded as protections against cold, in reality are unable to check heat loss for any considerable length of time. The rôle of these adaptations is a different one, namely, the protection against desiccation during the winter, which threatens the aerial parts of the plant as a result of the decreased water supply from the frozen soil. Frost resistance is not a capacity of dormant plants to protect themselves against the formation of ice, but a property of being very little or not at all affected by its formation.

This lack of susceptibility is attained due to chemical changes in the cells. It has been noticed for a long time that in the cold season leaves of plants, though wintering under snow, show no traces of starch. Considerable amounts of sugar are accumulated instead. Experiments have shown that sugar has the property of preventing the coagulation of albuminous substances by low temperatures. It therefore may be called a protective substance.

This protective influence of sugar may be readily demonstrated by means of the following simple experiment: Take three test-tubes containing the sap squeezed from a plant. This sap always contains a certain amount of protein. One of these tubes should be left as a check, the two others should be frozen, after a little sugar has been added to one of them. After thawing of the contents of both tubes, it is seen that in the tube which received sugar the proteins are in the same condition, a sol, as in the control.

The tube frozen without the addition of sugar will show a flaky precipitation of the coagulated protein (Fig. 48).

It is of interest to note that the sugar content in dormant plants is not constant. It increases with lower and diminishes with higher temperatures. The frost resistance of plants varies accordingly. It was noticed, long ago, that winter cereals when grown in warm

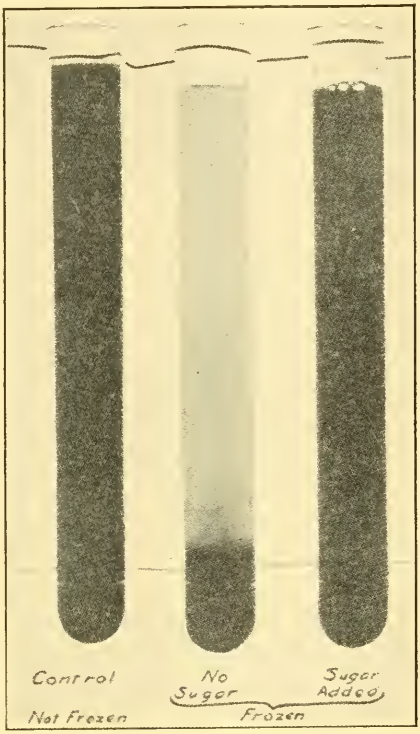

FIG. 48.—Protective influence of sugar in freezing expressed plant juice (*after Newton*).

greenhouses are easily killed by freezing, while those grown in the open show a high degree of hardiness. Even a prolonged thaw decreases perceptibly the cold resistance of plants. On the other hand, if plants grown in a warm place are kept for several weeks at a temperature but slightly above freezing, they may be hardened and may withstand a mild frost. Such hardening is always accompanied by an increase of the sugar content in the cells of

the plant. A similar accumulation of sugar may be observed in potatoes [1] stored at a temperature about 0° C., and the increase of sugars is readily perceptible to the taste. This unpleasant flavor is easily removed by keeping such sweetish potatoes for some time in a warm room.

Hardening, however, is not entirely due to the accumulation of protective sugar, the protein substances of the protoplasm also undergo changes, although these are not quite clear to us. It is known that irreversible coagulation sets in only at considerably lower temperature after hardening than before it. Especially marked seasonal variations in hardiness may be observed in trees. The needles of conifers, for instance, are able to withstand winter temperature of −30 to −40° C. while in summer they perish at −8° C.

Besides the accumulation of protective substances and the changes in the colloids of the protoplasm, increased hardiness is attained also by reducing the amount of free water. Dry seeds, containing not more than 10 to 12 per cent of moisture, are not susceptible to frost. They can stand without injury the temperature of liquid air and even that of liquid hydrogen. Soaked seeds perish at − 8 to − 10° C., and germinated seeds are still more susceptible to frost. The mature and dormant wood of branches of trees is much more frost resistant than the wood of growing shoots. In such desiccated tissues only a small amount of ice is formed even by severe freezing, hence they are little injured by frost.

In addition to the factors which increase frost resistance, there are also others which decrease it as, for instance, the abundance of free water or the acidity of the cells. Different tissues, and even separate cells of the same tissue, may show different degrees of frost resistance. In the seedlings of cereals, for instance, the most susceptible part is the plumule and the hardiest are the root tips and their central cylinders, while cortical parenchyma of the root is comparatively little frost resistant. After moderate freezing the root tips, therefore, continue to grow for some time while the young leaves and the external portions of the roots are killed.

Attempts to increase frost resistance artificially have not led so far to any positive results. The principal means of controlling

[1] But in this case, the ability to survive after freezing is not acquired.

the injurious effect of transitory frosts is by decreasing the power of radiation of the air. The air is either heated directly by means of portable stoves, or smudging is employed. In the latter case pitch, manure, dry leaves, etc., are burned in different parts of the orchard. These combustibles produce thick clouds of smoke which gather over the plants and thus decrease radiation. In order to prevent the damage wrought by prolonged winter frosts, one has to cover carefully sensitive plants with snow, straw, dry leaves, etc. Where this is impossible, as for instance with overwintering plants grown under field conditions, the only means of protecting is the selection of more hardy varieties. A detailed investigation of such hardier varieties has shown that in winter they possess a higher sugar content, less water, and a higher water-retaining power of their colloids.

CHAPTER V

ABSORPTION OF WATER

41. General Idea of the Water Balance. Amount of Water Absorbed by Plants.—In the preceding chapter it has been shown that to function normally the plant cell must be saturated with water. Naturally, no difficulty is experienced in this respect by submerged plants. For land plants, a constant supply of water from the soil is necessary. Most plant cells, especially those performing the work of assimilation, must be in close contact with air in order to obtain CO_2. As the air is very seldom saturated with water vapor, the water within the cell is continually given off. To make up for this loss, a constant current of water must ascend towards the cells.

Only a few of the lower plants, such as mosses and lichens, are able to stand a prolonged interruption of the water supply and to remain alive in the state of complete desiccation. For higher plants, such desiccation would result in death; hence, they possess a peculiar water balance. The absorption of water usually equals its loss. Plants adapt themselves to maintain this balance without persistent or serious deficit. Usually, they possess a well-developed root system which absorbs water from the soil with sufficient rapidity. A well-developed conductive system carries this water to the principal evaporating organs, the leaves. Finally, a series of protective adaptations checks the escape of water, if necessary.

The amount of water which passes through the plant is immense. Experiments have shown that during 1 hr. of a hot summer day more water passes through a leaf of our common plants than the leaf itself contains at any particular time. During the whole vegetative period, a sunflower or corn plant evaporates as much as 200 kg. (440 lb.) of water, *i.e.*, the contents of a barrel of considerable size (55 gal.) (Fig. 49). Of the total amount of this water, the plant assimilates but an insignificant fraction. This

becomes evident upon comparing the above figures with the amount of water entering into chemical reaction during the same period in the synthesis of carbohydrates. Under the environment of central Europe, for every kilogram of water absorbed only 3 to 4 g. of dry matter are formed. If it be assumed that the dry substance consists of carbohydrates and that half of this weight is made up by carbon, the other half consisting of the elements of

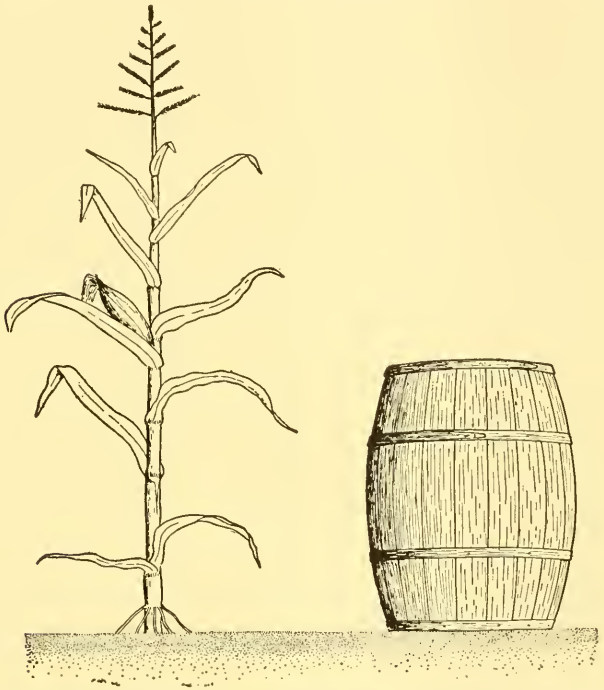

Fig. 49.—Amount of water evaporated during the summer by one maize plant (*after Sinnott*).

water, then it will be found that of the 1,000 parts of water that pass through the plant, only 1.5 to 2 parts are utilized in the process of nutrition. The remaining 998, or even 998.5 parts pass through the plant in order to compensate for evaporation and to maintain a sufficient degree of saturation in the tissues of the plant.

Such lavish expenditure naturally requires a no less rapid absorption of water. This task is performed chiefly by the roots. The real dimensions of the root system are much greater than most

people assume on the basis of such meager remains as are revealed when a plant is dug out in the usual way. The greater part of the most active fine rootlets are usually torn off in the digging. Rotmistroff, in Russia, and Weaver, in America, were the first to give a correct idea of the real extent of the root system, when all of its small branches remain intact. These authors have shown that even in cereals, as oats and wheat, the root system is not confined to the tilled layer. It penetrates into the soil to a depth of 1.5 to 2 meters and spreads extensively in all directions (Fig. 50). The roots of alfalfa and other plants of dry habitats penetrate considerably deeper. Even in small plants, the total length of the roots with all their branches measures several hundred meters. In larger plants this length has to be measured by kilometers. The absorbing surface of the roots is increased several times by the development of root hairs. Such an immense system enables the plant to utilize perfectly even a scantily distributed soil moisture and equally meagerly distributed mineral substances.

The enormous amount of water that is utilized by plants must not only be absorbed from the soil but it has to be conveyed to the evaporating leaves. Sometimes, especially in trees, the water current has to ascend to a height of several meters. The water distribution of the plant consists, therefore, of three principal processes: the absorption of water from the soil, its translocation to the place of consumption, and its loss in the process of transpiration. Each of these three processes will be discussed separately.

42. Laws Governing the Entry of Water into the Cell. ' Suction Tension and Methods of Its Determination.—The absorption of water by the cell from the surrounding medium is determined by the osmotic properties of the cell. If a plant cell is immersed in water, first the colloids of the cell wall and then the protoplasm become saturated with water. The protoplasmic sac containing the cell sap checks the passage of the substances dissolved in water, but allows the latter to pass through. Therefore the cell sap absorbs water (see Art. 36), and, increasing in volume, exerts a pressure on the protoplasm, and through the latter on the cell wall.

If the cell walls were of unlimited extensibility, this absorption of water by the cell would continue until the concentrations of the inner and outer solutions became equal. Distending under the influence of the entering water, the cell wall exerts on the cell contents an elastic counterpressure, opposite in its direction to

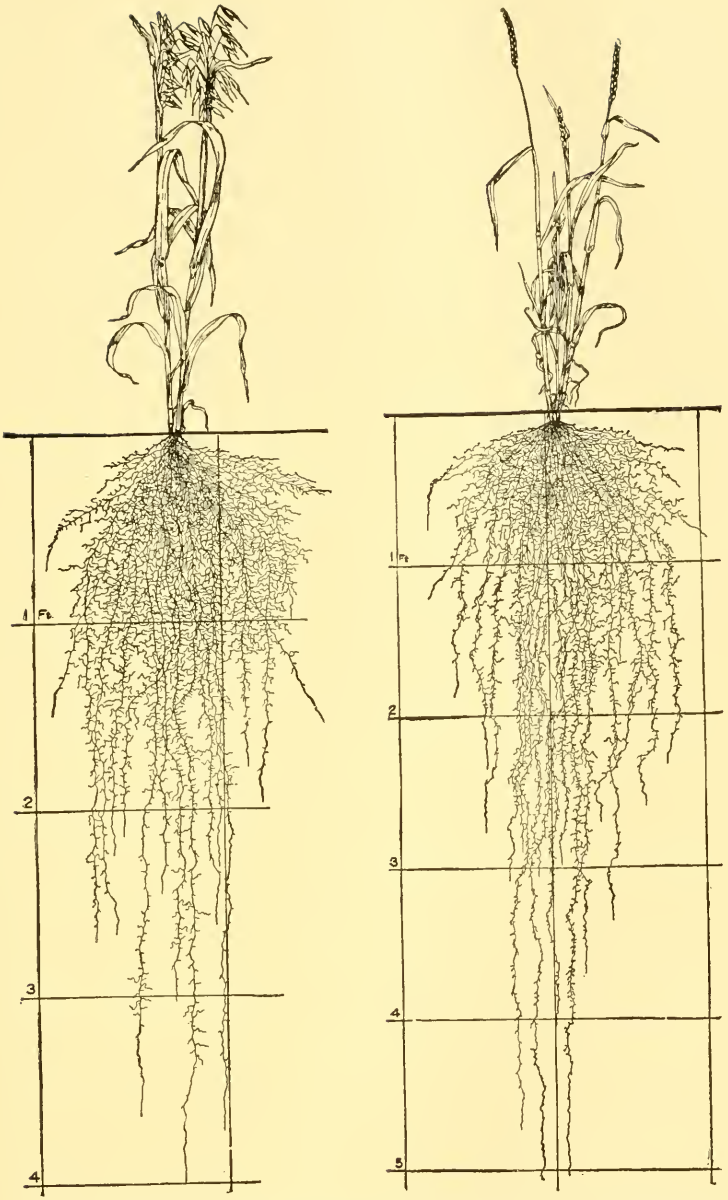

FIG. 50.—Oats and wheat in the flowering stage, with a root system carefully removed from the soil. The depth is shown in feet (*according to Weaver*).

the osmotic pressure and tending to counterbalance it. With further increase of volume, the pressure of the cell wall on the cell contents is augmented. Finally, the moment comes when the pressure of the wall equals the osmotic pressure and as a consequence a further increase in volume ceases. This condition of equilibrium may be called the state of complete saturation of the cell with water. If P denotes the osmotic pressure of the cell sap, and T the pressure of the wall on the cell's contents, as a result of its strained condition which is called "turgor pressure," it will be in the state of complete saturation with water, $P = T$, or, otherwise, $P - T = 0$. The water will neither enter the cell nor escape from it, no matter how great the sap concentration within the cell might be. This condition of perfect saturation is natural to the cells of submerged plants, but it is hardly ever found in land plants. Because of the loss of water in the process of transpiration, their cells are never quite fully saturated with water and, hence, do not attain perfect turgidity. Part of their osmotic pressure remains unbalanced by the counterpressure of the cell wall. When such a cell is immersed in water, this additional internal pressure induces the entry of water into the cell, and consequently leads to an increase in volume. This enlargement continues until the increased turgor pressure equals the magnitude of the osmotic pressure. For land plants, therefore, our formula reads as follows:

$$P > T \quad \text{or} \quad P = T + S$$

or, finally,

$$P - T = S.$$

The value S has been called by Ursprung "suction tension." It is this value, and not the absolute magnitude of osmotic pressure, that determines the absorption of water by the cell. As may be seen from the above formula, this value is the difference between the full osmotic pressure of the cell sap and the turgor pressure of the distended cell wall.

Not every cell has the same suction tension because this depends on its water saturation. The lower the state of saturation of a cell or the more water it has lost in the process of transpiration, the higher will be its suction tension. Thus the plant cell possesses a self-regulating osmotic mechanism which absorbs water the more vigorously, the more it is in need of it. In the

condition of wilting, or of complete loss of turgor, the suction tension reaches its maximum. In this case it is equal to the magnitude of the osmotic pressure of the cell sap, as may be seen from the formula $S = P - T$, where S is the greater the smaller T is, and S has the highest value when $T = 0$.

In order to determine the magnitude of the suction tension Ursprung has devised a method somewhat similar to the plasmolytic one. The cell is immersed in solutions of different concentration, whose osmotic pressure approaches the suction tension of the cell. In solutions whose osmotic pressure is greater than the suction tension, the cell loses water and decreases in volume. In weaker solutions, on the contrary, the cell imbibes water and increases in volume. Only in a solution whose osmotic pressure equals the suction power of the cell does the volume of the latter remain unaltered.

Ursprung's method, though simple in principle, requires painstaking work when put into practice. Before immersing the cell into any of the solutions, its volume must be measured, for which purpose an ocular micrometer is used. To prevent the desiccation of the cell during the time of measuring, it is immersed in liquid paraffin, which is harmless and checks evaporation. With sufficient attention and careful manipulation, the method gives excellent results. For rougher and more rapid determinations of suction tension, instead of single cells, whole sections of tissues may be used. The length of these sections is measured before and after immersion in the test solutions. The solution in which the length of the section does not change, is equal in its osmotic value to the suction tension of the cell.

The relationship between turgidity, osmotic pressure, and suction tension may be graphically illustrated by the following diagram (Fig. 51). On the abscissa are marked the various stages of tension of the cell wall at different degrees of saturation of the cell with water, or at different volumes of the cell. On the left, is seen the condition of a cell when completely plasmolyzed or wilted and, therefore, at its smallest volume, which is denoted by 1. The concentration of the cell sap, and consequently its osmotic pressure 0, attains its maximum. The suction tension, then, equals the osmotic pressure. When the cell imbibes water, its volume, and consequently the degree of tension of the cell wall, increases, and a gradually increasing turgidity T sets in. When the cell sap

becomes diluted with water and its osmotic pressure falls, the suction tension rapidly decreases. On the right side of the diagram is illustrated the condition of complete saturation with water, the suction tension having fallen to zero and the turgidity now balancing the entire strength of the osmotic pressure of the cell. Under the usual "average" conditions of a cell, denoted in this diagram by the letter *G*, a part of the osmotic pressure is balanced by turgor pressure. The other unbalanced part represents the magnitude of the suction tension.

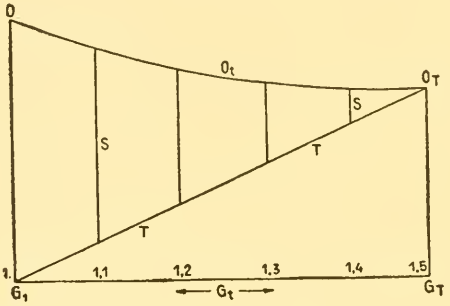

FIG. 51.—Diagram showing changes of the osmotic value in a cell passing from saturation with water to wilting (from right to left), and *vice versa* (from left to right): *T*, turgor pressure; *O*, osmotic pressure of the cell sap; *S*, suction tension; *G*, degree of distention of the membrane (*after Höfler*).

43. Suction Tension and Exudation Pressures of the Root System. Root Pressure, Guttation, and Bleeding of Plants.—Land plants of simple structure, for example the mosses and lichens, absorb water through their whole surface. In higher plants, special organs, the fine tips of roots or rootlets, are provided for this purpose. The cells of this part of the roots show thin, unprotected walls. In the majority of cases they are provided with special outgrowths, the root hairs, which greatly increase the absorbing surface of the plant. Root hairs penetrate into the soil capillaries and absorb the water contained therein.

If the plant did not lose water continually in the process of transpiration, the root cells would soon be saturated with water and absorption would cease. The loss of water by the plant must, therefore, be regarded as the principal process inducing an unsaturated condition in the plant and promoting the formation of considerable suction tension. The plant as a whole presents, as it

were, a self-regulating osmotic apparatus. The suction tension, originating in the leaves is finally transmitted through a series of mechanisms to the root system, where it creates a water deficit which is necessary for the initiation of suction.

Arising in the plant as a result of the physical process of evaporation, this saturation deficit may be called "passive suction." The root system possesses a means of creating this deficit in its cells in a purely physiological way, because of the water-expelling activity of the cells of roots. The water is driven in a definite direction, namely, through the cells of the cortex into the vessels of the xylem, which are situated in the central cylinder. The course of the water current ascending in the root may be represented by the following diagram (Fig. 52). Into cell *A*, provided with a root hair, the water is absorbed from the soil and moved on to cells *B*, *C*, etc., until it reaches cell *L*, adjoining vessel *M*. Here it is driven into the vessel and ascends the stem.

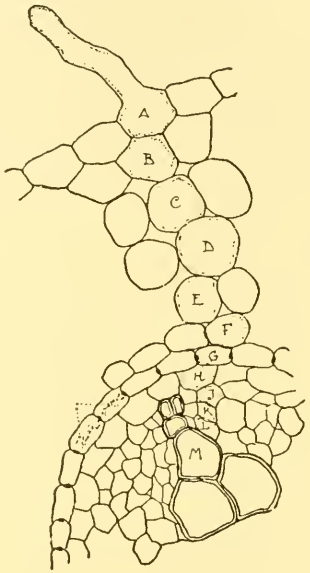

Fig. 52.—Path of water from the root hair *A* to the vessels of the root *M* (*after Priestley*).

To observe the active pressure of the root cells, a plant is cut above the soil. To the cut stump a rubber tube is fastened ending in a glass tube. The water excreted through the cut surface accumulates and rises in the glass tube. If the latter is bent down and a vessel placed under it, the water exuding from the root system may be gathered and analyzed (Fig. 53). This phenomenon has been called the "bleeding" of plants. The force that drives the water into the vessels is termed "root pressure." To measure this force, a mercury manometer is connected to the cut stump of the plant.

The phenomenon of bleeding is exhibited by various plants in different degrees. In some, as *Fuchsia*, nettle, sunflower, it is readily detected. In others, for instance the conifers, it is almost imperceptible. Bleeding shows also seasonal variations. It is

most profuse in spring, especially in woody plants, when in some

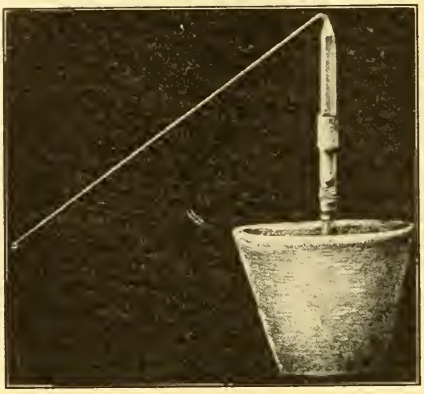

FIG. 53.—Exudation of sap from a cut stem as a result of root pressure (*after Molisch*).

of them, as the birch, maple and grape, the sap flows abundantly from the cut stems and branches.

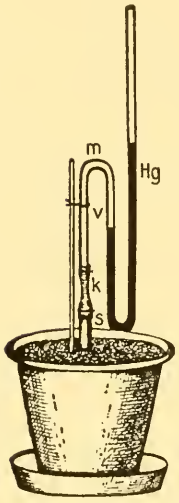

FIG. 54.—Measuring root pressure by means of a mercury manometer (*after Molisch*).

Root pressure may be observed likewise in uninjured plants. Seedlings of cereals, when placed in a moist atmosphere, accumulate water drops on the tips of their leaves. From time to time these drops run down and are replaced by new ones. Similar drops may be observed at the margins of leaves of nasturtium (Fig. 55), *Fuchsia*, or *Alchemilla*, and on the leaves of the potato and buckwheat. This phenomenon is known as "guttation." It is produced by the same root pressure that drives the water through the vessel of the leaf, just as through a glass tube. Especially strong guttation may be observed in *Arum*, *Colocasia*, and *Philodendron*, plants commonly cultivated indoors. Under favorable conditions, such as high temperature, and high air and soil moisture, the tips of their leaves may drip continually.

The amount of water excreted under the influence of root pressure is, on the whole, insignificant. It does not exceed a few cubic centimeters per day.

The pressure developed in this process is also small. In herbaceous plants it usually constitutes but a fraction of an atmosphere. Only in some trees and in the grapevine, has a pressure above 1 to 2 atmospheres been observed. It must be remembered, however, that such experiments are usually conducted under conditions where the root system is separated from the aerial parts of the plant and, therefore, may soon weaken. As the water in the manometer accumulates but slowly, the recorded pressure is considerably lower than actually exists. Indirect determinations of the root pressure, recently carried out by Sabinin have given greater values. In some herbaceous plants, such as corn, wheat, and *Impatiens*, the bleeding pressure has been found to approach 1.5 to 2 atmospheres.

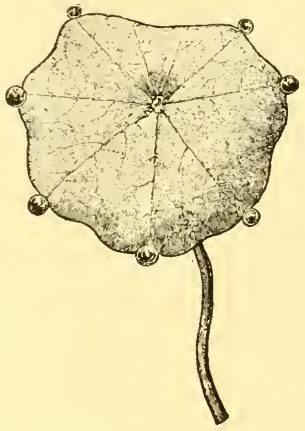

FIG. 55.—Guttation of a nasturtium leaf (*after Noll*).

44. The Osmotic Mechanism of Root Pressure.—The pressure of the root cells, as revealed by the phenomena of bleeding and guttation, is closely connected with their life activity, but in particular with the unalterable condition of their osmotic properties. A simple experiment may serve as an illustration. A small pot with strongly guttating wheat seedlings is placed under a bell jar filled with vapors of ether or chloroform. Watering of the soil with a solution of some poisonous substance may serve the same purpose. After a time the root cells will be killed and guttation will cease. The same results may be obtained by killing the roots with heat, or by depriving them of oyxgen.

This result is easily understood, since it has been seen that all osmotic properties of the cell are closely dependent upon the normal structure and function of the protoplasmic membrane. With the death of the cell this structure undergoes profound changes. The cell loses its semipermeability, along with its turgidity and suction pressure. The dependence of root pressure on the life activity of the plant, however, has been found to be very complex. Not only a normal unaltered condition of the physicochemical properties of the protoplasmic membrane is required, but likewise an

uninterrupted supply of nutrient substances. If this supply is checked, as may be observed in the usual experiments with the guttation of plants, when the aerial organs are cut, bleeding rapidly decreases, or ceases.

To understand the cause of this phenomenon, the mechanism of root pressure will be examined. It has been seen that the water enters the root by virtue of its osmotic suction, and, having passed through a series of parenchyma cells it is driven with considerable force into the vessels. From the osmotic system of the cell, however, no conclusion can be drawn as to the reasons for the possible expulsion of water from the cell. Although Dutrochet, who was the first to investigate osmotic phenomena in plants, compared the rise of the sap in the vessels of roots and stems to the rise of the liquid in the tube of an osmometer, the similarity is not very great. The tube of the osmometer is the direct continuation of its cavity. The rise of the liquid, therefore, is simply the result of the general increase in volume of the same uniform solution filling the osmometer. In the root, on the contrary, the cavities of the vessels are separated from the adjoining cells through which the water is driven. Besides, it is not the cell sap but a considerably more dilute solution, that ascends through the vessels. In order to visualize root pressure, it must be accepted that at least some root cells are able simultaneously to imbibe water and to expel it into the vessels. Therefore, the osmotic mechanism of the cell already discussed, must be somewhat modified in order to understand this flow of water in one direction.

Fig. 56.—Guttation of the sporangiophore of *Pilobolus* (*adapted from Lepeschkin*).

For this purpose several modified mechanisms have been suggested. At present, it cannot be said with certainty which of these is correct. The theory proposed by Lepeschkin seems to have gained the widest recognition. This theory is based on the supposition that an unequal permeability of the protoplasm exists in different parts of the cell. Lepeschkin was led to this idea by his observations on the water drops exuding on the surface of the sporangiophores of a fungus, *Pilobolus* (Fig. 56). It is well known that these fungi possess no septa within their hyphae. From an osmotic point of view, therefore, their whole body represents, as it

were, a single cell. When immersed with its lower branches in a moist substratum, this cell imbibes water and expels it from its upper parts. By means of plasmolysis, Lepeschkin has demonstrated that the protoplasm of the sporangiophore actually shows a somewhat greater permeability than that of the absorbing hyphae. On the basis of this observation, he has constructed a theory of pressure in one direction. In places with less permeable protoplasm, the pressure on the plasmatic membrane by a solution of the same concentration will be greater than at points with a more permeable protoplasm. In the latter instance, part of the pressure is lost, due to the diffusion of some of the dissolved substance through the protoplasm, since, according to the laws of hydrostatics, the elastic counterpressure exerted on the protoplasm by the distended membrane is everywhere the same, and on the whole equals the average magnitude of the pressure of the solution on the membrane. The result will be that in places of less permeability the water is imbibed owing to the excess of the inner pressure over the outer while in places showing greater permeability, the water is pressed out through the protoplasmic membrane. Thus, an uninterrupted water current proceeds in one direction through the cell.

The fluid exuding from the cell is not pure water, but a solution somewhat more dilute than the cell sap. The whole mechanism depends upon and works at the expense of substances leached out from the cell. This explains the necessity of supplying the cell with nutritive substances from other parts of the plant. A physical model of such a pumping cell may be constructed in the following way: A glass cylinder, open at both ends, is filled with a sugar solution. One end of the tube is covered with a more permeable membrane than the other, for instance, animal bladder and vegetable parchment. To the more permeable end a rubber cap is fastened, ending in a glass tube in which the exuding sap accumulates (Fig. 57). If this model of a cell is immersed in water, the same pressure in one direction will be observed, as is exhibited by *Pilobolus*.

In addition to that of Lepeschkin, another idea, tending to explain the flow of water in one direction, has been proposed chiefly by Priestley and recently has gained wide recognition. This scheme is based on the consideration that the water-absorbing cell, being saturated, develops no suction by itself. This is in

perfect harmony with the experimental data of Ursprung, who has found that the suction tension of root hairs, when immersed in water, is equal to zero. But the solution which is present in the cavities of the dead tracheal elements as a result of the excreting activity of the living cells adjoining the vessels, is not subject to the pressure of the impermeable protoplasm. At this point the solution, therefore, possesses a suction tension equal to the whole magnitude of its osmotic pressure. It must absorb water from

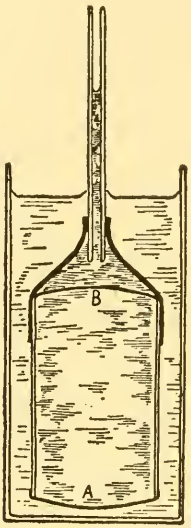

FIG. 57.—Hofmeister's model illustrating the pumping of water by a cell: *A*, less permeable membrane of animal bladder; *B*, more permeable membrane of parchment paper.

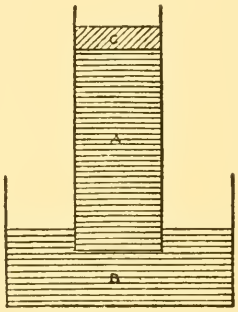

FIG. 58.—Diagram of water current passing through the cell in one direction (*according to Priestley*).

the adjoining saturated cells, which begin to show a decrease in saturation or suction tension. This loss is compensated for at the expense of the surrounding solution. It tends to prove, therefore, that it is not the living cells that draw water from the soil, but rather the solution present in the vessels. The whole aggregate of living cells only filter the fluid but remain otherwise passive. The mechanism of this suction may be respresented by the following simple diagram (Fig. 58). One end of cell *A* is immersed in a vessel of water *B*. On the other end is poured a solution *C* of a certain concentration. Since cell *A* is sat-

urated with water, solution *C* will draw water through it, quite independently of the magnitude of the osmotic pressure in cell *A*.

Priestley's scheme naturally requires that solution *C* come in contact with vessel *B* through cell *A*. Hence, there can be no diffusion of the solution through the cell walls. But the walls of cells are commonly made up of cellulose which is readily permeable to diffusing substances. Priestley, therefore, supposes that the rôle of an impermeable septum is played by the endoderm whose corky radial walls seem to be formed for this function.

This scheme explains quite cleverly the mechanism of root pressure, as well as the rôle of the endoderm in certain parts of the root, the function of which before this seemed to be a puzzle. It also allows the measurement of the suction power of roots and the determination of the concentration of the solution exuded from the cut stump of bleeding plants. According to Priestley's idea, the concentration of this solution controls suction tension. The determinations by Sabinin, already referred to, have shown that the suction tension of the roots of our common crop plants approximates 1.5 to 2 atmospheres; at any rate, it cannot be much above this value.

45. Absorption of Water from the Soil. The Water-retaining Forces of the Soil. Available and Unavailable Water.—The absorption of water from the soil presents a much more complicated and difficult problem than the simple imbibition of water by roots immersed in a glass container. A number of forces are found in the soil which counteract the suction of the roots. The sum total of all the forces that retain water in the soil may be called the water-holding capacity of the soil.

It is not pure water but a solution of a certain concentration that is found in the soil and, therefore, it shows suction tension. If for convenience Priestley's supposed mechanism is adopted, then it can be readily seen that with an equal concentration of the solution in the soil and in the vessels of the plant, all suction must cease. But here, likewise, a special regulating mechanism exists in the plant, which indicates that the higher the concentration of the soil solution, the greater also is the concentration of the solution in vessels of the plant. This mechanism, however, works only within certain limits. The majority of plants are unable to grow in strongly saline soils, where the concentration of the soil solution

is very high. In such places a special flora develops, the so-called "halophytes." These plants show the faculty of accumulating an enormous amount of salts and develop a very high osmotic pressure, up to 100 atmospheres or more.

Besides this osmotic resistance to suction, another type of resistance due to adsorption is found in the soil. The soil consists chiefly of sand particles of different size together with more or less decayed organic matter of a colloidal nature. Inorganic colloids also are present. The water, moistening the soil, is connected in various degrees with these principal parts of the soil. Some of the water fills the larger spaces in the soil and remains in a rather mobile condition. This is called ground water. It obeys the force of gravity, descending into the soil after a rainfall. In the smaller soil capillaries, the water is retained by the forces of surface tension. It does not follow the force of gravity. This water may rise in the soil, though slowly, above the level of the ground water. It is called capillary water. The force by which capillary water is retained in the soil is small, usually a fraction of an atmosphere, depending on the diameter of the capillary. This water, therefore, is absorbed by the root hairs without difficulty, usually by the surface cells of the absorbing zone of the root. Ground water, of course, is absorbed still more readily.

Water directly surrounding the soil particles is in a different state. This water is retained by the force of molecular attraction, or adsorption. This force is of a considerable magnitude. Moreover, it increases rapidly as the water film surrounding the soil particle grows thinner. Such a film of water is available to the plant only with difficulty. Finally, air-dry soil contains, from 0.5 per cent in coarse sand, to 14 per cent in heavy clay, of so-called "hygroscopic" water. Hygroscopic water is retained by the soil particles with a force reaching as high as 1,000 atmospheres and may be unavailable to the plant.

The colloidal substances of the soil have the property of swelling in water. They develop considerable water-holding capacity. The more of these colloidal substances are present in the soil, the more water is bound to them. The amount of this so-called "imbibitional" water is especially great in peat soils, which consist almost entirely of partially decayed plant residues.

In the soil the root hair has to compete for water with the

surrounding soil particles. The smaller these particles are and the more colloidal substances are present in the soil, the greater is the amount of water retained and unavailable to the root cells. This competition for water by the plant has been formulated by Sachs. His diagram showing the absorbing root hair among the water-retaining soil particles is presented here (Fig. 59).

To determine the amount of water in the soil that is unavailable to the plant, the following procedure may be used. When the plant under investigation has developed sufficiently, watering of the soil is stopped and the pot is kept in a shaded place until the plant begins to wilt. Wilting indicates that the water supply from the

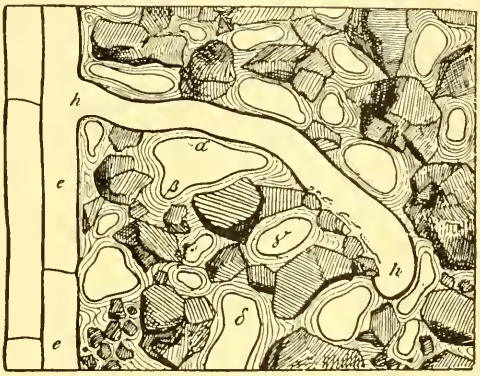

FIG. 59.—A root hair in the soil, absorbing water held by soil particles.

soil has ceased. At the moment of permanent wilting the amount of water that still remains in the soil may be measured by taking a sample of the soil and drying it at 100° C. This is the reserve unavailable to the plant.

The most detailed investigation of the amount of unavailable water in different soils was carried out by Briggs and Shantz. They investigated numerous samples of various soils and established a simple relationship between the amount of unavailable water which they called the wilting coefficient and the other water-holding properties of the soil, for instance, hygroscopic capacity and the total water-holding capacity, as well as its mechanical composition. From their data, these authors were able to make the following formulæ for the calculation of the wilting coefficient q,

or the unavailable moisture reserve of the soil, in percentages of its dry weight.

$$q = \frac{\text{per cent of hygroscopic water}}{0.68}$$

$$= \frac{\text{per cent of water at full saturation} - 21}{2.9}.$$

According to the data of mechanical analysis, the unavailable reserve may be calculated as follows:

$$q = \text{per cent sand} \times 0.01 - \text{per cent silt} \times 0.12 - \text{per cent clay} \times 0.57$$

Thus, different soils show a very different wilting coefficient. For coarse sand, this coefficient is 1 per cent; for fine sand, 2 to 3; for loam, 5 to 10; for heavy clay soil, 14 to 16 per cent. Regarding the adaptability of different plants, it has been found that with sufficiently slow wilting, all of them leave in the soil a perfectly equal amount of water, though the osmotic suction tension of their root systems may be very different. This fact may be explained as follows: When the capillary water, which is readily available to all plants, is exhausted, the water-retaining forces of the soil rapidly increase to several atmospheres. As the suction tension of the plants usually does not exceed a few atmospheres, differences in this regard between various plants play almost no rôle. Moreover, the water loses its capacity of moving through the soil; hence, the soil particles which are not in direct contact with the root hairs no longer give up their moisture to them, however great the suction tension of the root hairs may be.

The data by Briggs and Shantz are in perfect agreement with those of Bogdanov, obtained at a much earlier date by studying the relation of germinating seeds to soil water. Bogdanov has found that, independently of their nature and properties, the seeds of various plants can germinate only when the water content of the soil is no less than twice its hygroscopic capacity. His method of calculating the unavailable water has been made use of in agronomic practice. In calculating the amount of soil water available to the plant, usually twice the amount of hygroscopic water is subtracted from the total amount present. Under conditions of rapid evaporation, the amount of unavailable water is

somewhat greater, almost three times the hygroscopic capacity of the soil.

46. Influence of Environmental Conditions on the Absorption of Water. Methods of Studying the Absorption of Water by Roots.—One of the environmental factors which strongly influences the rate of absorption of water by the roots is undoubtedly the temperature of the soil. This was detected long ago by Sachs, by means of very simple and illustrative experiments. He noticed that such potted plants as tobacco, beans, or pumpkin, rapidly show signs of wilting when the pot is surrounded with ice. When

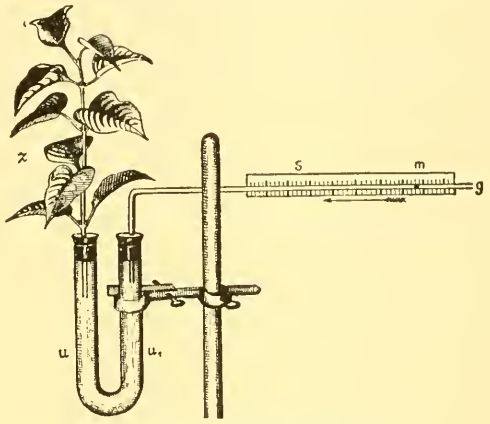

FIG. 60.—Potometer (*after Molisch*).

this is removed and the soil is heated, the plants recover without additional moisture. From this, he drew the conclusion that under the influence of low temperature the rate of root absorption slows down, so much that it is no longer able to make good the loss sustained by evaporation. Such a primitive method, however, is not sufficient for the exact determination of the amount of water absorbed by the plant. Special apparatus, called "potometers," are now used, which allow a quantitative determination of the volume of water absorbed (Fig. 60). The essential parts of a potometer are: (1) a container through the upper end of which a plant, inserted in a stopper, is introduced in such a way that the root system is within the container; (2) a graduated capillary tube placed horizontally for observing the amount of water absorbed. Otherwise, a potometer admits of an almost endless number of

modifications. Frequently, only branches instead of whole plants are employed. It is often expedient to construct a potometer that can be weighed, in order to determine the water lost by the plant.

Exact quantitative determinations which have been carried out by means of potometers have shown that with a lowered temperature the rate of water absorption by the roots rapidly slows down. This reduction, however, is not equal with different plants. The thermophilous plants of the tropics and subtropics show a reduction in their suction power more rapidly than do plants of temperate zones. The impeded absorption, caused by low temperatures, is induced not so much by the slowing down of the diffusion rate, which changes but slightly under the influence of temperature, as by the altered properties of the protoplasm. At low temperatures, the viscosity of the liquid part of the protoplasm increases. It may even congeal, as a solution of gelatine does at room temperature. Such congealing slows down markedly the rate with which water passes through the protoplasm. Experiments have shown that not only the water absorption of a whole root system, but also plasmolysis and deplasmolysis connected with the passing of water through the protoplasm, are strongly retarded by a low temperature. At 0° C. this process is only one-quarter to one-seventh times as fast as at 20° C.

The retardation in water absorption by a cooled soil explains many peculiarities in the life of plants growing in temperate zones. In autumn, when the temperature of the air is still quite high during the daytime and transpiration is considerable, the roots absorb water only from the already cooled soil. As a result, a considerable water deficit may arise within the plant. The usual response by a plant to these conditions is a reduction of its evaporating surface. This is effected by abscission of leaves through the formation of an abscission layer across the petiole. According to Schimper, cold soil is physiologically dry, though it may be saturated with water. This explains many peculiar structures of bog plants, which evidently are intended to lessen transpiration, since bog soils are cold and thaw slowly in the spring. Many bog plants, as *Oxycoccus*, *Andromeda*, and *Ledum*, have leathery leaves with somewhat involute margins. Their stomata, through which water is given off are depressed in tubes. This structure helps to decrease transpiration. Other bog plants, for instance *Eriophorum*, have almost awl-shaped leaves.

The influence of a low soil temperature on the absorption of water by the plant, however, must not be overestimated. In hardy plants, as for instance winter cereals, low temperatures interfere less with the intake of water. These plants grow and develop perfectly well in late fall and early spring, in spite of periodical frosts. Their unhindered absorption of water from the soil is indicated by an abundant guttation.

Oxygen of the air is another external factor influencing the rate of water absorption by the root. In order to develop exudation pressure, as well as for their growth, roots require a supply of oxygen. On too compact or submerged soils, therefore, plants develop poorly, or perish. This is frequently observed in small depressions of the ground where water stagnates. It is not the excess of water itself that is injurious to the plant, since it develops perfectly in water cultures. It is rather the lack of oxygen resulting from submersion that is harmful. Plants in water cultures develop successfully only when an air current is passed through the solution or the latter is changed often. Experiments with potometers have resulted in a delay of absorption, where the water was saturated with carbon dioxide or nitrogen, thus removing the oxygen. The inhibiting influence of carbon dioxide was more marked than that of nitrogen, the former apparently acting as a specific poison.

Besides the accumulation of the poisonous carbon dioxide, lack of aeration may lead to other consequences having an indirect effect on the root system. The various bacterial processes of the soil, for instance, may be profoundly altered. Oxidation may be replaced by anaerobic fermentation. Poisonous products of anaerobic decay may accumulate in the soil, which in American literature have been termed soil toxins. All these substances poison the roots of plants and check water absorption. The usual herbaceous vegetation does not develop, therefore, on very swampy soils. In these places grow special plants, whose roots are in a way immune to the soil toxins. Moreover, submerged and bog plants have air spaces in their organs. These air spaces help to supply with oxygen the roots and rhizomes, which are usually surrounded by mud.

Owing to this adaptability, bog plants thrive in soils which are entirely lacking oxygen and poisonous to other plants. The abundant guttation, so characteristic of many of these plants, shows

that their roots develop a normal suction and exudation pressure.

47. Absorption of Water by Aerial Parts of Plants.—The root system is the special organ of water absorption. This does not mean, however, that the other organs of the plant are unable to absorb water. Every unsaturated cell imbibes water, if brought into contact with it. Thus leaves, especially when wilting, absorb water vigorously upon immersion, in spite of their cutinized epidermis. Experiments have shown that a dry cuticle is almost impermeable to water. When moistened, it swells and becomes much more permeable.

Leaves moistened by rain or dew are able, therefore, to imbibe water. The dormant branches and buds of trees likewise absorb water through their surface during thaws and spring rains. This absorption, however, plays no important rôle in the general water régime of the plant. The amount of water absorbed always remains small in comparison with the total amount of water lost. Moreover, the moistening of the cuticle increases the intensity of tranpiration, thus accelerating the loss of water. This explains the well-known rule that the watering of plants in direct sunlight must be avoided, as it induces more rapid wilting. Cautious watering of the soil without sprinkling the leaves, however, does not produce harmful consequences, even when done during the noon hours, if the water does not lower too much the temperature of the soil.

Only for some plants, whose existence is subject to the humidity of the air, the absorption of water by the aerial organs is an important means of water supply. Such are the so-called "epiphytes," plants living on the surface of other plants or rocks, especially on the trunks of trees. In the climate of Russia, only lower plants are found in this group, chiefly lichens and some mosses. When it rains, these plants absorb water with their whole surface and swell considerably. During periods of drought, they lose all this water and may become perfectly dry without losing their vitality. As soon as they are moistened they again return to activity. Their protoplasm, consequently, is capable of reversible desiccation.

In the tropics, especially in moist regions with almost daily rainfalls, epiphytism is rather common among higher plants, for instance, the orchids and the *Bromeliaceae* in general. In these plants a considerable amount of water is stored in the thickened

leaves, but more especially in the tubers. This water reserve is spent very slowly during the drier periods. To facilitate the absorption of atmospheric moisture, such plants possess special adaptations. Many orchids have long aerial roots, hanging down (Fig. 61) or trailing over the bark of trees. These roots are covered by several layers of empty cells with perforated walls, which absorb water like a sponge. The water is then transmitted to the inner living tissues of the root. This spongy tissue is termed *velamen* (Fig. 62). In their structure, the cells of this tissue remind one of the empty cells in the leaves of sphagnum moss, which also serve to retain water. In

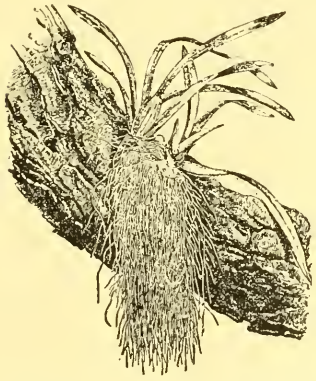

Fig. 61.—A tropical orchid with aerial roots.

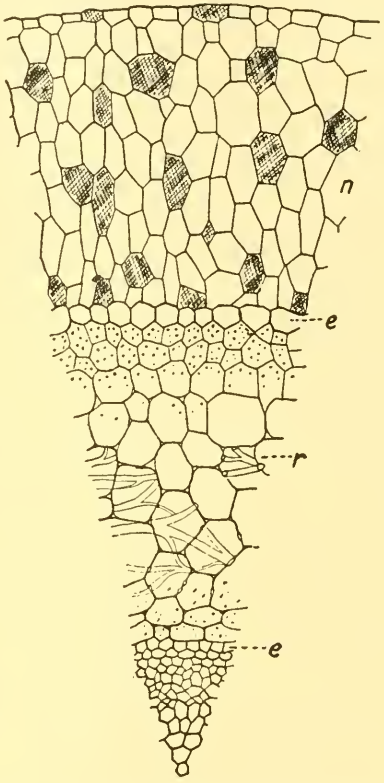

Fig. 62. — Cross-section through the root of an orchid. From epidermis to *e*-velamen (*after Nathansohn*).

Bromeliaceae, on the leaves are found valvelike scales, which, being appressed to the epidermis during dry weather, prevent the escape of water. During rains, the cells forming the stipe swell, the scales are raised and allow the entrance of water. In addition, the leaves of these plants are arranged in such a way that in their clasping bases the rainwater is continually and gradually absorbed.

All these adaptations serve for the absorption of water in liquid form. Indications that plants are able to absorb water vapor are based on erroneous observations and are nowadays refuted by the majority of investigators.

48. Absorption of Substances Found in the Soil Solution.— The root hairs, and from these all other parts of the plant, absorb the substances present in the soil solution along with water. It would be erroneous, however, to think that a plant, like a simple

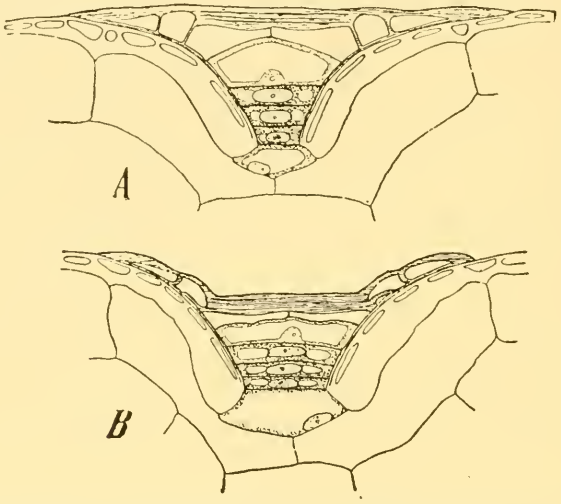

FIG. 63.—Absorption scale of a bromeliaceous plant: *A*, in swollen condition *B*, during drought (*after Nathansohn*).

wick, passively absorbs all substances dissolved in the soil solution. Before entering the vessels of the plant, the soil solution has to pass through a series of living cells. These, being endowed with selective permeability, produce considerable changes in the solution.

Experimental evidence based on the composition of plants, and consequently the amount of soil minerals accumulated by it have shown that absorption of soil nutrients depends to a higher degree on the specific properties of the plant itself than on the composition of the soil solution. Thus, the potato will always accumulate potash salts; the pigweed and nettle, potassium nitrate; and cereals, silica. The composition of the nutritive solution used in

sand cultures must be chosen, therefore, according to the requirements of the plant. If this is not taken into account, the plant may absorb various substances in an unequal degree and the composition of the solution may change considerably with the growth of the plant. Even with perfect adaptation to the character of the plant, one does not succeed in preparing a solution that would be absorbed unaltered. Sand cultures, therefore, are more difficult to manage than water cultures, where the solution may be changed from time to time.

Not only the composition, but also the general concentration of the solution changes measurably as the plant develops. If it is too dilute, the plant draws from it relatively more salts than water and it gradually becomes more diluted. From strong solutions, on the contrary, the plant absorbs proportionately more water than salts, resulting in an increased concentration of the solution, which finally leads to decreased growth. The absorption of water and of salts, thus, to a certain degree is independent of each other. This is shown by experiments in which transpiration is artificially checked by placing the plants in a moist atmosphere or by shading them. The total amount of the water lost thereby is considerably reduced. The amount of accumulated salts, on the other hand, remains almost unchanged.

The most direct way of determining in what proportion water and the substances dissolved in it are absorbed by the plant, is by the analysis of the sap excreted by plants in bleeding; with the exception of spring bleeding, when the exuding sap is extremely high in reserve substances. Such determinations have been carried out recently by Sabinin, who has shown that the composition of sap does not correspond to the liquid in which the root system is immersed. If kept in very dilute solutions of K, P, Mg, and other substances, the plant is able to concentrate considerably these elements in its sap. On the contrary, when placed in strong solutions, the concentration of the sap excreted in bleeding is relatively low. There is no doubt that this condition is due to the membrane equilibrium of Donnan, mentioned in Art. 38.

Substances incapable of penetrating through the protoplasm or unable to accumulate in the cells are absorbed by the roots from the soil in rather insignificant quantities. An alien substance, for instance a dye or an insecticide, cannot be introduced into the conductive system of a plant by watering the soil with a solution

of such substance or even by placing the roots of the plant in the solution. It is necessary to open the conductive system and to let the solution directly into the cut vessels. Nutritive substances may be introduced in the same way, as for instance iron salts in the case of chlorosis. In order to inject solutions into a tree in this radical way, a funnel may be fastened to the tree. An opening is made penetrating the bark and part of the wood. Through this opening the solution is absorbed in unaltered condition and soon reaches the leaf cells. Herbaceous plants also may be made to absorb solutions in this extraordinary way. Especially adaptable for such experiments are plants with broad vessels, such as the different representatives of the *Cucurbitaceae*.

CHAPTER VI

THE LOSS OF WATER BY THE PLANT

49. Transpiration. Its Rôle in the Life of a Plant.—Land plants lose considerable amounts of water through evaporation from the surface of their cell walls. Evaporation is essentially a physical process in which water changes from a liquid to a gaseous state in an unsaturated atmosphere, and then diffuses as vapor into the surrounding space. The mechanism, however, is considerably complicated by a number of anatomical and physiological peculiarities of the evaporating surface. Hence it must be really considered a physiological process. It leaves a marked effect on many phases of the life of land plants and is responsible for a whole series of other related physiological processes. The evaporation of water by the plant is usually designated by the special term "transpiration."

Transpiration is a physical necessity for all terrestrial plants; but, in instances of great dryness of the air and lack of water in the soil, a continual loss of water may lead to harmful and even fatal consequences. Emphasizing this, some authors (Schwendener, Timiriazev) have come to believe that transpiration is nothing more than an unavoidable evil. This, however, is not entirely true. Under normal conditions of growth, the loss of water by the leaves is readily replaced from the soil. Moreover, the process of transpiration is very likely the major cause of translocation of water towards the evaporating leaves. No great "injury" really can be caused by transpiration. Also the absorption and upward translocation of mineral salts is facilitated by the water current which continually passes through the plant. Another favorable consequence of transpiration is the reduction in temperature of the leaves, which enable them to function even in the brightest sunlight without injury. Experiments have shown that the temperature of wilting leaves, whose transpiration is reduced, is 4 to 6° higher than of turgid ones, a difference which sometimes may prove fatal to the

plant. In greenhouses and hotbeds where the moist air lessens transpiration, overheating and severe burning of the leaves has been noted. Another physiological result of transportation has been pointed out by L. Ivanov. According to this author, a certain saturation deficit in the cells is necessary for the normal function of a plant, especially for its flowering and fruiting. This optimum degree of turgor is maintained by transpiration. All these considerations compel one to believe that transpiration is not an unnecessary evil, but that it is a very important physiological process.

One must not suppose, however, that in order to function normally a plant has to transpire as much water as is usually lost under natural conditions. Observations and experiments have shown that this amount greatly exceeds the necessary minimum. Transpiration can often be considerably reduced not only without injury, but even with advantage to the plant. It has been noted, that the amount of salts absorbed by a plant is by no means proportional to the amount of water transpired, and later it will be shown that the drier the climate and the higher transpiration, the more water is used for the production of a certain quantity of organic matter. But no sharp distinction can be made between useful and excessive loss of water.

50. Methods of Studying Transpiration and Units of Measurement.—The methods for the study of transpiration are comparatively simple. They can be divided into three groups:

1. The collection and determination of the evaporated water.
2. The determination of changes in weight of the plant resulting from loss of water during transpiration.
3. The determination of the amount of water absorbed by the plant to compensate for that lost by transpiration.

In order to determine the amount of water vapor transpired, the plant under investigation is placed in a container. The latter is usually of glass, in order not to exclude light which plays an important rôle in transpiration. The eliminated water is collected by means of some hygroscopic substance. Under simple arrangements a plant is put into an airtight container, together with a cup containing some water-absorbing substance, such as calcium chloride, phosphoric anhydride, or sulphuric acid. The increase in weight of the cup shows the amount of water trans-

pired. In stagnant air, however, transpiration is retarded. It is more expedient, therefore, to draw a current of air through the vessel containing the plant. This air, however, must pass through calcium chloride tubes or an apparatus containing some other water-absorbing substance (Fig. 64). The advantages of this method are that the leaf or branch under investigation remains attached to the plant. This is almost the only method that can be used in determining the transpiration of trees under natural

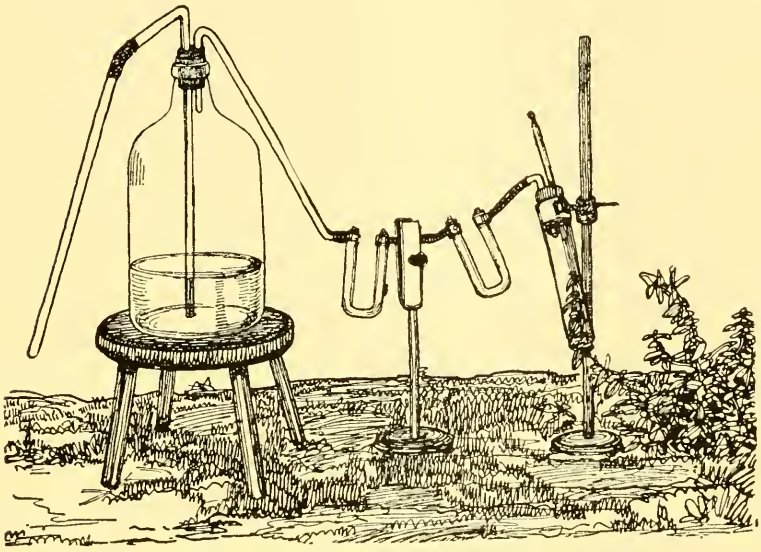

FIG. 64.—Apparatus for the study of transpiration (*redrawn after Freeman*).

conditions. Its drawback is that the transpiring organs are enclosed in a container, which must undoubtedly interfere with the normal course of transpiration.

Somewhat analogous to the quantitative methods, which are based on the increase in weight of a water-absorbing substance, is the so-called cobalt-chloride method, in which the color changes exhibited by filter paper impregnated with a solution of cobalt chloride are used to indicate the transpiration rate. When dry, this paper has a blue color; when moist, it becomes pale pink. If a slip of such paper is placed on the transpiring surface of a leaf and then covered with glass, its color will change. The more rapidly this change takes place, the higher the rate of transpira-

tion. By this simple method, it can readily be demonstrated that there is a considerable difference in the rate of water loss from the upper and lower surfaces of leaves which have their stomata mainly or wholly on one surface. The cobalt-chloride method is a qualitative one. But, during recent years, Livingston and his coworkers have made an endeavor to raise it to the level of a quantitative method by measuring with a stop watch the time in which the blue paper turns pink.

The most reliable methods of studying transpiration are those based on changes in weight of the plant resulting from loss of

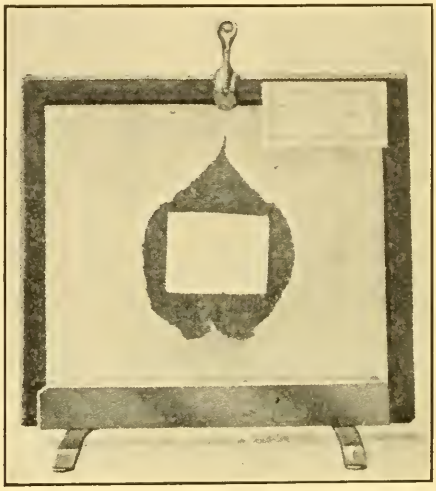

Fig. 65.—Cobalt chloride method of measuring transpiration (*after Nathansohn*).

water. It may be supposed that such methods might introduce a considerable error, for changes of weight depend not only on the loss or gain of water, but also on the gain or loss of dry substance, due to assimilation and respiration. Numerous experiments have shown, however, that the weight of water lost by a plant per unit of time is several hundred times as great as the increase in dry weight. The error resulting from not taking into consideration assimilation and respiration is therefore negligible.

For the study of transpiration, whole plants rooted in soil, or cut branches, or leaves may be used. Rooted plants permit the study of transpiration under more natural conditions, but the necessity of weighing such plants together with the soil in which

they grow introduces a series of technical difficulties. The plants either have to be grown in special containers impermeable to water vapor (Fig. 66), or, at the time of the experiment at least, the pot must be enclosed in some metal or rubber covering, in order to prevent evaporation from the surface of the soil. For more exact experiments special transpiration balances (Fig. 67) combining high sensitivity with large capacity have to be used.

FIG. 66.—Galvanized-iron pot with closely fitting cover for the exact determination of water used by a plant.

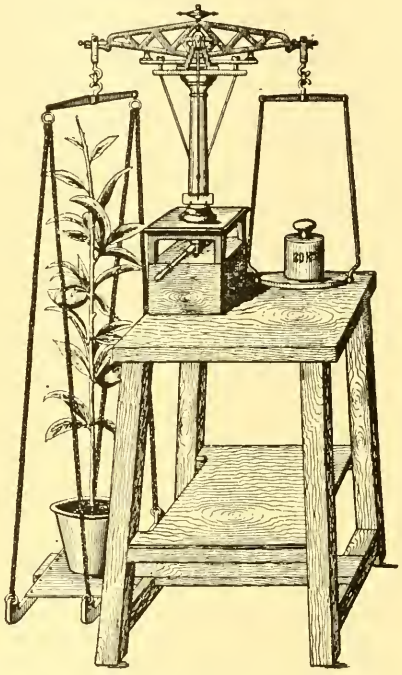

FIG. 67.—Transpiration balances (*after Burgerstein*).

Experiments with cut shoots and leaves are more convenient as they remove the necessity of weighing heavy pots of soil, which must inevitably affect the sensitiveness of a balance, hence, this method of studying transpiration is widely used. The shoot is usually placed with its cut end in water, so that the water lost may be continually replaced. Precautions must be taken to prevent the evaporation of water otherwise than through the shoot. This is accomplished by inserting the twig through a hole in the

stopper of the container (Fig. 68). Air is permitted to enter the container through a glass tube inserted in the same stopper. In experiments of a short duration with slowly transpiring plants, cut parts from a plant are weighed without the water. In either case, however, cutting is not without an influence on transpiration. Even when placed in water immediately, cut portions of a plant may wilt rapidly in direct sunlight. Frequently, cut leaves and shoots transpire more rapidly, when placed in water, than do those remaining on the plant, since they may have a better water supply. If one tries to calculate the total amount of water given off by the tree from the transpiration of a single leaf, and the number of leaves on a tree, he runs the risk of obtaining abnormal values. Experiments with cut organs of the plant, therefore, give no reliable data as to the magnitude of their transpiration under normal conditions.

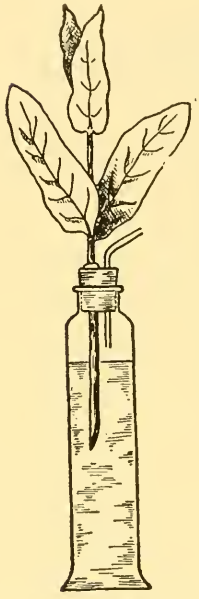

FIG. 68. — Method of determining the water used by a shoot.

Still less reliable results are obtained by methods that are based on the determination of the volume of water absorbed by the plant, as when different kinds of potometers are used, to which attention already has been called in Art. 46. Potometers are useful for purposes of demonstration. They have one advantage: the readings may be taken uninterruptedly. But the amount of water absorbed does not always coincide with the amount of water transpired. Even during a limited period the error may be as high as 50 per cent. In exact experiments the gravimetric method of determining transpiration is to be preferred to the volumetric.

In order to obtain a continuous record of the march of transpiration, self-recording mechanisms called "transpirographs," are used. In the majority of cases, these are transpiration balances in which the movements of the hands or the scales is in some way transmitted to a revolving drum with a smoked surface. Experiments with transpirographs are usually successful only in a closed room, such as a greenhouse. When used out-of-doors, the accuracy of the records is interfered with by wind.

In order to compare the results obtained by different experiments, it is necessary to reduce them to a common standard unit. Most frequently the total quantity of water lost by a plant during a certain period is expressed per unit of its leaf surface. The magnitude thus obtained is called the "intensity of transpiration" and is usually expressed in grams per hour per square meter of leaf area. Sometimes, instead of the use of the surface which in highly dissected leaves of irregular shape is difficult to determine, the green, or even the dry weight of the leaves is used in calculation.

In comparing the amount of water lost per unit time with the total amount of water contained in the plant, the *rate of expenditure of the water reserve*, expressed in percentage, is obtained. Comparing the amount of water lost by the plant during a rather long period, several weeks, or even the entire growth period, with the amount of dry matter accumulated in this time, the *transpiration efficiency*, expressed in grams per kilogram of water lost, is obtained. The reverse value, the number of grams of water used in accumulating 1 g. of dry substance, is termed the *transpiration coefficient*. Some authors, chiefly American, call this value the "water requirement of the plant."

A picture of the intensity of transpiration is obtained by comparing the rate of transpiration from a unit leaf surface with the rate of evaporation from a unit area of a free water surface. The magnitude obtained is called *relative transpiration*. It shows to what extent the transpiration of a leaf is slower when compared to "free evaporation."

All these magnitudes vary greatly with different plants and under the influence of different environmental conditions. In most plants, the intensity of transpiration ranges from 15 to 250 gm. per square meter per hour in daytime, and from 1 to 20 g. at night. The rate of water expenditure may fluctuate from 10 to 80 per cent; the efficiency of transpiration from 1 to 8; under the conditions of a moderately moist climate it is frequently 3. The transpiration coefficient correspondingly varies from 1,000 to 125. Most often, it is 300. Relative transpiration is commonly expressed by values of 0.1 to 0.5, sometimes almost attaining 1, and in other cases falling to 0.01 or below, especially in plants well protected from water loss.

51. The Physical Nature of Transpiration. Its Dependence on Environmental Conditions and Its Daily March.—Essentially,

transpiration represents the physical process of evaporation and as such may be expressed by Dalton's formula.

$$V = K(F - f)\frac{760}{P}S,$$

where K is the coefficient of diffusion or evaporation, F the saturation pressure of water vapor in air at the temperature of the evaporating surface, f the observed vapor pressure in the surrounding space, P the barometric pressure, and S the area of the evaporating surface. From this formula it follows, in the first place, that transpiration is proportional to the saturation deficit of the air; not to the relative moisture, as is often erroneously considered. Second, the formula shows that with a rise in temperature the rate of transpiration goes up, for then the value of F increases very rapidly.

Evaporation from areas of small dimensions shows considerable deviations from Dalton's rule. According to Stephan's law, referred to in Art. 11, evaporation from a small surface is proportional not to its area, but to its diameter. Other conditions being equal, several small leaves lose more water than a large one of equal area. The greatest deviations from Dalton's law may be observed when the air is perfectly still, which is rarely realized under natural conditions. As soon as wind comes into play, these differences become considerably smaller. In general, wind markedly increases evaporation and thus transpiration by drawing the moist air from the surface of the leaf and as a consequence from the intercellular spaces within the leaf.

Light has a very important function in the process of transpiration. Since chlorophyll absorbs easily radiant energy, the temperature of the leaf is increased perceptibly in light. This immediately increases the difference between the factors F and f and consequently intensifies transpiration, which in turn lowers the temperature of the leaf; hence, plants that have a high power of evaporation are hardly heated at all in light, but their transpiration increases considerably. It was stated in the chapter on assimilation (Art. 10) that usually only 1 to 3 per cent of the radiant energy absorbed by the plant is used in photosynthesis, while over 80 per cent is spent in transpiration. Even diffuse light increases transpiration by 30 to 40 per cent; while in direct sunlight it may

be multiplied several times. This accounts for the great difference between day and night transpiration, which is many times higher than a similar difference in evaporation from any transpiring surface.

Besides the direct heating effect, light increases transpiration also indirectly. It favors the opening of stomata and increases the permeability of the protoplasm of evaporating cells. Both of these circumstances favor the loss of water by plants.

The influence of environmental factors controls the daily march of transpiration. It is low in the early morning hours, increases rapidly with the rise of the sun, and the rise in temperature, and increases the saturation deficit, attaining its maximum in the early afternoon and again falling rapidly with the setting of the sun. If curves are plotted for the diurnal changes in transpiration, solar radiation, the saturation deficit, and temperature, it will be noted that these curves will almost coincide. Radiation, however, reaches its maximum somewhat ahead of transpiration, while the highest point in the saturation deficit is attained somewhat later (Fig. 69). This shows that sunlight is the chief factor determining the intensity of transpiration.

A typical daily trend in transpiration is naturally observed only on absolutely cloudless days, when meteorological conditions are normal. It will show sharp fluctuations in changeable weather, largely in accordance with changes in temperature and light.

52. Leaf Structure as a Factor in Transpiration. Stomatal and Cuticular Transpiration.—The rate of transpiration is determined not only by external factors, but to a high degree likewise by the structure of the leaf, as well as the state of its cells and tissues during transpiration. These factors complicate considerably the process of water loss.

Evaporation proper, that is, the transition of water from a liquid to a vaporized condition, takes place at the surface of the parenchymatous cells lining the intercellular spaces of a leaf.

These open spaces represent a special aeration system, the outlets of which are the stomata. The rest of the leaf is covered by a solid layer of epidermal cells the outer walls of which are coated with a cuticle very little permeable to water or water vapor (Figs. 11 and 80).

The cuticle not being entirely impermeable to water, it does not completely stop transpiration. This may be demonstrated by

coating with vaseline the lower surface of such leaves which have no stomata on the upper side, leaves of many trees are best for this purpose. These leaves will continue to transpire, although at a reduced rate. This loss of water through the cuticle is called "cuticular transpiration," as distinguished from "stomatal transpiration" in which water escapes through the stomata. In fully developed leaves stomatal transpiration is 10 to 20 times as rapid

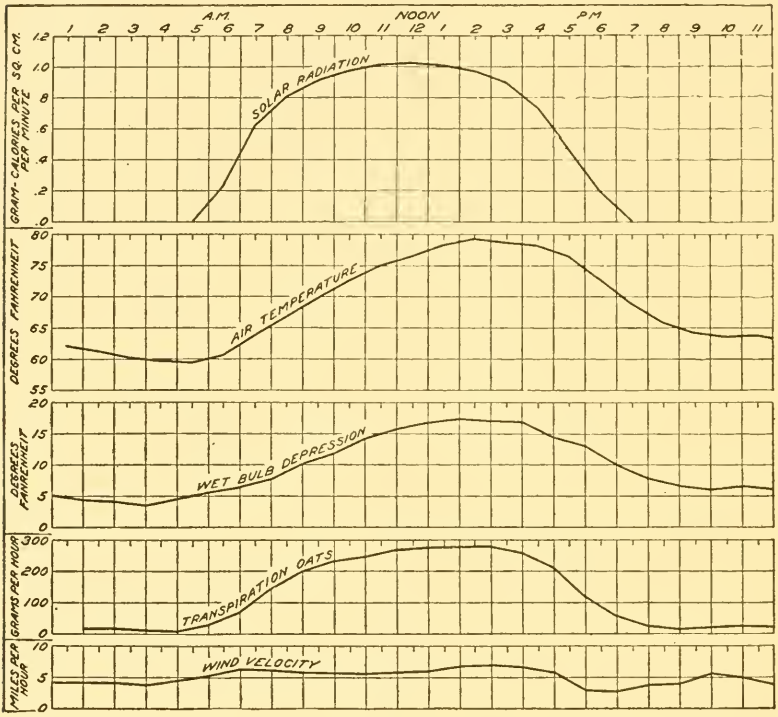

FIG. 69.—Graphs showing the daily march of transpiration and the most important factors determining it (*according to Briggs and Shantz*).

as cuticular transpiration. But in young leaves, with cuticle, not completely developed, as well as in leaves growing in the shade or in a moist atmosphere, half of the water may be transpired through the cuticle. Moistening of the leaves also increases considerably cuticular transpiration, as was mentioned in Art. 47.

Stomatal transpiration consists of two phases: the evaporation from the surface of the water-saturated mesophyll cells lining the

intercellular spaces, and the diffusion of the vapor thus formed through the stomata. The laws governing diffusion through stomata have been discussed previously, and it has been shown that the great number of these very small openings in the cuticle strongly favors diffusion. This process may go on through the stomatal openings, constituting only 1 to 2 per cent of the leaf area, almost at the same rate as if the cuticle did not exist and the internal cavities were fully open to the air. A diagram illustrating this is shown in Fig. 70. Comparative determinations of the intensity of transpiration from a leaf and of evaporation from a free water surface have shown that this condition may be realized. In some very vigorously evaporating plants, the relative transpiration may attain the magnitude of 0.8 to 0.9; while under favorable conditions 0.4 to 0.5 are average figures. If transpiration were proportional to the area of the openings, then the values would not exceed 0.01 to 0.02. Thus we see that, per stoma, the diffusion rate of water vapor is very high.

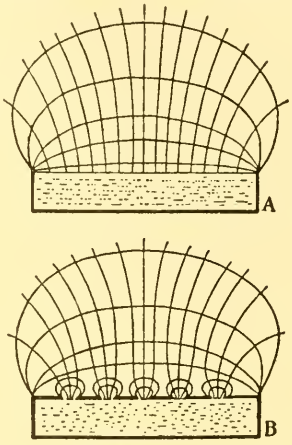

FIG. 70.—Diagrams showing diffusion of water vapor from an open vessel *A* and through a multiperforated membrane *B* (*after H. Walter*).

The diffusion capacity of the epidermis supplied with innumerable stomata is so high that the plant seldom uses it to full capacity. This is true at least for the process of transpiration. The stomata play also an important rôle in assimilation, as they are the openings giving access to carbon dioxide. Actually, transpiration is always considerably lower than its calculated value. Wherefrom, the conclusion may be drawn that besides changes in the stomatal openings, other factors may exert a checking influence on transpiration. These factors will be discussed later.

53. The Stomatal Apparatus of the Plant. Methods of Studying Stomatal Movements.—One of the most important peculiarities of the stomata is their ability to change the diameter of the opening. Light and the water content of the leaf are the chief external factors responsible for this. In the majority of plants, the stomata are opened in light and closed in darkness.

With high water content of the foliage they are usually open; with lack of water, closed.

The mechanism which controls stomatal movements is a very peculiar one and not yet fully understood in all its details. It is subject to the transformation of starch into sugar, which takes place in the guard cells and is regulated by enzymatic processes. When starch is transformed into sugar, the sap concentration, and consequently the suction tension of the cell, increases. When guard cells imbibe water, their volume increases and their curvature changes, resulting in their separation and a widening of the opening between them. But when sugar changes into starch, the reverse processes take place and the stomata close (Fig. 71.) This connection between the movement of stomata and the transformations of starch has been clearly established by Lloyd and Iljin. It is of interest to note that the influence of external factors on the transformation of starch in the guard cells and in other cells of the leaf is very different. In the mesophyll cells, starch is formed in light and dissolved in darkness. In guard cells, on the contrary, starch disappears in light and is again accumulated with its absence. Lack of water leads to the transformation of sugar into starch and the stomata close. With a sufficient amount of water in the cell sap, sugar accumulates and the stomata are opened.

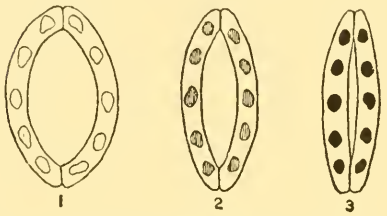

FIG. 71.—Changes in the starch content of guard cells during their movements (*after Iljin*).

Since stomatal movements are controlled by a rather complex mechanism, their response to changes in the environment is also rather complex. Under extreme influences, as very severe wilting, or a rise of temperature above 40°, as well as the action of Na and K cations, the mechanism undergoes striking disturbances. The hydrolysis of starch then rises rapidly, while the converse process, the transformation of the surplus of sugar into starch, is entirely suppressed. This causes a great increase in osmotic pressure of the guard cells. They become saturated with water, open abnormally wide, and often lose the capacity to close again. Under extreme dryness of the air this may lead to a complete desiccation of the leaf. According to the investigations of Zalensky, such

abnormal opening of the stomata is observed during hot desiccating winds, which explains their harmful effect.

The mechanism of stomata may be studied in various ways. The simplest method would be the direct observation under the microscope of stomatal movements in an uninjured leaf. But on account of their small size and the lack of transparency of the leaves, this method may be used only with few plants. Moreover, it is inconvenient to work with a microscope in a field or forest. The method proposed by Lloyd is therefore used more frequently. According to this procedure, a strip of epidermis is quickly removed with forceps and plunged into absolute alcohol. This instantly dehydrates the cell walls and preserves them in the condition they were in at the moment of stripping. Such a fixed preparative may be kept indefinitely, and examined under the microscope whenever necessary.

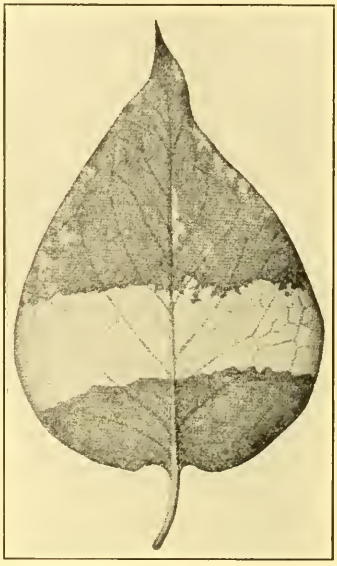

Fig. 72.—Transparent band formed after the infiltration with benzol across a part of a leaf that was illuminated and therefore had open stomata (*after Molisch*).

A still simpler method was devised by Molisch. It is based on the fact that liquids capable of wetting the cuticle will readily penetrate the open stomata and rapidly fill the intercellular spaces of the leaf. Such an injected leaf becomes quite transparent. In order to determine the degree of opening of the stomata, a drop of benzol or of absolute alcohol is placed on the leaf by means of a small brush or glass rod. If the stomata are open, the drop is rapidly absorbed and transparent patches appear on the leaf (Fig. 72). If they are closed, the drop will dry rapidly without leaving any trace. As benzol has the capacity to penetrate into narrower openings than are penetrated by alcohol, it may infiltrate a leaf with nearly closed stomata. By applying both liquids, alternately, one is able to determine not only whether the stomata are open or closed, but also the approximate degree of opening. Instead of

benzol and alcohol other liquids, such as benzine, kerosene, etc., may be used. The infiltration method is not exact, but it is simple and well adapted for use under natural conditions.

For determining the degree of opening of the stomata, Darwin devised a special apparatus called by him the "porometer." It consists of two parts: a bell jar about 1 cm. in diameter, and a T-shaped tube (Fig. 73). The tiny bell jar is glued with its flange to the leaf surface. This leaf chamber is connected by a rubber tube with one of the horizontal arms of the T-tube. The other arm ends in a rubber tube which is controlled by a clamp, while the vertical part of the tube dips into a vessel of water. When air is sucked through the rubber tube and the clamp then closed, the air within the tube and bell jar becomes rarefied and the water is raised in the vertical arm. If the stomata are open, the air enters through them into the bell jar and the water column in the tube falls. The rate of falling indicates to some extent the degree of opening of the stomata. When they are completely closed, the water column remains stationary for many hours at the height to which it was raised.

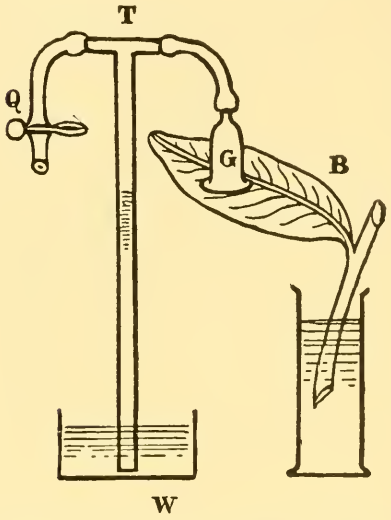

FIG. 73. — Darwin's porometer, showing arrangement of the parts of the porometer (*after Darwin*).

By using any of the described methods, the daily march of the stomatal movements may be observed. It has been noted that in bright but not too dry or hot weather there are fairly regular diurnal changes. In the majority of plants the stomata open at dawn, or even earlier. The maximum opening is usually attained in the morning hours. About noon the stomatal slit begins to narrow slightly, and it closes usually a little before sunset. When the sky is overcast, the openings are not so wide as in bright weather. In very dry weather the slits open in the morning but soon close again, as a result of loss of water by the leaf. Fre-

quently about noon, or between 10 and 11 o'clock in the morning the stomata may have closed completely. Under conditions of maximum heat and dryness they remain closed the whole day long and open only for a short time during the early morning hours.

The behavior of stomata varies with different plants. In some, as the potato, cabbage, beet, etc., they are usually open. Under

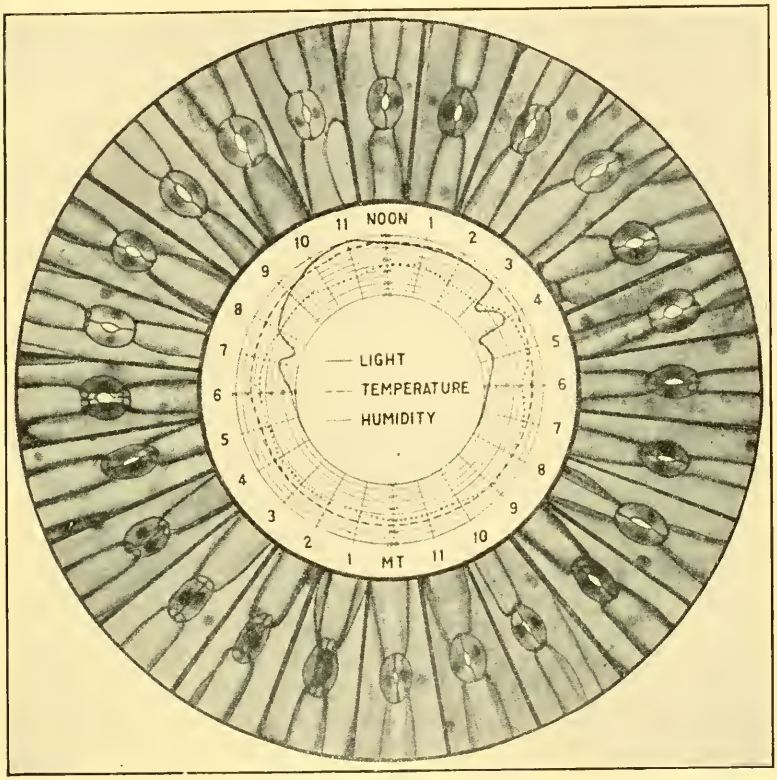

FIG. 74.—Daily march of stomatal movements in the onion. Within the circle are graphs showing diurnal changes in light intensity, temperature, and humidity (*after Loftfield*).

optimum conditions of water supply they tend to remain open throughout the day and night. In other plants, for instance the cereals, stomata are always closed at night. Moreover, they close very early in the evening and at the slightest deficiency of moisture may become shut in the morning hours. The majority of plants occupy an intermediate position in this behavior (Fig. 74). It is

interesting to note that in plants showing stomata on both sides of the leaf, those on the upper surface open later and for a shorter time. Likewise, the stomata near the tip of the leaf open earlier and close later than those at the base.

54. The Regulation of Transpiration.—The transpiration process consists of two phases: evaporation proper, which takes place in the intercellular spaces; and diffusion of the water vapor through the stomatal openings into the surrounding air. The intensity of transpiration is usually under the controlling influence of two kinds of factors: changes in the diameter of the stomatal pores, and changes in the degree of saturation of the cell walls.

Since changes in the degree of opening of the stomata may be observed directly, the stomatal movements were, until recently, regarded as the only means of regulating transpiration. This controlling influence of the stomata is based on the fact that when the stomata are closed, outward diffusion of water vapor is entirely stopped. The air in the intercellular spaces then becomes saturated and water is no longer evaporated, thus permitting only cuticular transpiration to take place, which is comparatively insignificant. The marked decrease in transpiration at night, mentioned in Art. 51, depends not only on environmental conditions but also on the fact that in the majority of plants the stomata are completely closed at night.

But the fact that the complete shutting of stomata stops transpiration, does not permit the conclusion that simply a narrowing of the stomatal pore will lead to a corresponding check in water loss. The epidermis with its stomata may be viewed as a multiperforated septum. Because of the small dimensions and great number of these openings, diffusion takes place almost at the same rate as if a septum did not exist (Arts. 11 and 52). Within certain limits, changes in the stomatal pores, therefore, induce a slowing down of the diffusion rate. An exact correlation between the degree of opening of the stomata and the intensity of transpiration has not yet been established. Recent investigations, however, make it very probable that a decrease of 50 to 75 per cent in the diameter of a stomatal pore does not seem to influence transpiration very greatly. A further narrowing of openings, however, will result in a perceptible check in water loss.

Light, which plays such an important rôle in transpiration, promotes the opening of the stomata. They close in light only with

great difficulty. This influence of light on the movements of stomata may seem incomprehensible as regards water economy. It is understandable, however, when we remember that the stomata are the channels through which carbon dioxide must enter the leaf, and for successful photosynthesis it is requisite that the stomata be open during the hours when the plant is illuminated by the sun. The closing of the stomata during the daytime, though lessening transpiration, would have an unfavorable effect on the nutrition of the plant.

Another way of regulating transpiration would be by a reduction of evaporation from the surface of the cell walls within the intercellular spaces. Such decrease may take place only when the cell walls lining the intercellular spaces become drier than usual. Such a dehydration of the walls in turn is subject to a lower degree of saturation of the cells themselves, and, consequently, an increase in their suction tension. As a result of this mechanism, based on the equilibrium between the suction tension of the cell contents and the colloidal cell walls, a water shortage in the leaf, due to excessive transpiration or an insufficient water supply from the soil, is in itself a cause of decreased transpiration. Whether this is accompanied by a narrowing of the stomatal pores or not is of small moment. This phenomenon has been called "incipient drying" (Livingston). It must be regarded as a most important non-stomatal regulator of transpiration.

The degree of moisture in the cell walls depends not only on the condition of saturation of the cell, but likewise on the permeability of its protoplasm. If the protoplasm is quite impermeable to the substances in the cell sap, then the osmotic pressure of the latter will attain its highest value, and water will be drawn with a maximum force from the cell walls. If the permeability of the protoplasm increases, the pressure of the sap on the protoplasm, and through it on the cell walls, diminishes. Part of the water is pressed out of the cell by the contracting cell wall, the surface of the wall is covered with water, and transpiration increases. This mechanism is especially conspicuous when cells die, and as a consequence the protoplasm loses its semipermeability. Hence, leaves killed by frost or heat, or merely injured in this way, lose water very rapidly, and become dry. Minor fluctuations in permeability, such as are effected by the influence of light or narcotics on leaves, likewise accelerate water loss from the cells.

55. Wilting and Its Physiological Significance.—When the loss of water considerably exceeds the supply, a shortage of it ensues in plant cells, and their volume and turgidity diminishes. A considerable water deficit may lead to a complete loss in turgidity. In such cases the plant wilts, *i.e.*, its tissues lose their normal rigidity and the leaves and young tips begin to droop. If water is supplied in time to such a plant, its turgidity as well as its normal life activities will be restored.

Two types of wilting are distinguished: a temporary and a permanent one. The first is observed when transpiration increases to such an extent that the water supplied by the soil is no longer able to replenish the loss. The leaves, as the organs spending most of the water, lose their turgidity and wilt, while elsewhere in the plant the moisture content still may be quite high. When towards the evening transpiration decreases, the deficit is made good and the plant usually recovers without additional watering of the soil.

Permanent wilting happens when the soil no longer contains water available to the plant. No matter how slow the loss of water may be under such conditions it always results in a gradual loss of turgor of all organs of the plant, including the root hairs. The latter, being especially sensitive to a shortage of moisture, are destroyed easily, which breaks the close connection between the root and the soil. After permanent wilting, the plant absorbs water very slowly even from a very moist soil. Only after the renewal of the root hairs is the former rate of water supply to the plant restored. Moreover, with the desiccation of the chlorophyll-bearing cells of the leaf, the green plastids are injured. They lose for a long time their faculty of assimilating carbon dioxide. Permanent wilting proves detrimental to the nutrition of plants.

Permanent wilting is also harmful to the other organs and tissues of the plant, the growth of the newer parts commonly being retarded. If wilting occurs in the early stages of fruiting, ripening and filling of the grain is irregular, the yield is reduced, and the grain itself is poorly developed. In some plants, like the cotton, abscission of the flower buds may be observed. Such results, though of less intensity, are produced also by temporary wilting.

Although definite harm is done to the plant by wilting, especially when permanent, it should not be regarded as a pathological phenomenon. Experiments have shown that in plants undergoing

wilting the loss of water is markedly reduced by the closing of stomata as well as by the drying of the cell walls. Thus protecting itself the plant loses only one-fifth to one-tenth as much water as in a condition of turgidity. Wilting must be regarded, therefore, as a most effective way of checking transpiration in periods most dangerous for plants.

Apparent wilting, or the visible loss of turgidity, is shown by various plants at different degrees of water loss. Plants grown in open places, as the potato or the sunflower, may lose 25 to 30 per cent of their total water content without showing signs of wilting. Such water loss has been actually observed in these plants during the midday hours on all hot days. On the other hand, shade-loving plants may wilt with a loss of 2 to 3 per cent of water. These differences are due to the fact that in plants of the first group the cell walls are much distended, similar to a blown rubber balloon. Part of this tension may be preserved even with a considerable decrease in volume. In the second group, the walls may be strained but not distended. Even a small decrease in volume makes them collapse like a paper bag.

The capacity to resist wilting, even when the water loss is considerable, is to the advantage of a plant. The stomata will remain open and the plant will be able to continue its work of assimilation.

56. The Conservation of Water of Desert Plants. Xerophytes, Mesophytes, and Hydrophytes.—Water is very unequally distributed over the surface of the earth. There are places where plants have always an ample supply of water at their disposal. But there are also large tracts of desert with a very scanty vegetation, a few isolated shrubs scattered over a naked soil. The plants of such dry habitats, where the soil contains but little water and the air is hot and dry, have been called xerophytes. The opposite type are the hydrophytes—plants characteristic of perpetually moist habitats, as near a body of water or in a tropical rain forest. The majority of plants of temperate climates and of moderately moist habitats are of an intermediate type, the mesophytes. A large majority of crop plants belong to this group.

Since none of these ecological types are represented by a homogeneous group, they cannot be characterized by a few definite physiological features. This is especially true of the xerophytes—plants having adapted themselves to very severe habitats. Though

exhibiting a great diversity in their adaptation, one character is common to all xerophytes; they exhibit a small transpiring surface. This becomes especially conspicuous when compared with the comparatively large underground organs. Xerophytes are usually herbaceous plants or dwarfy shrubs, in which the underground parts are many times larger than the aerial organs (Fig. 75).

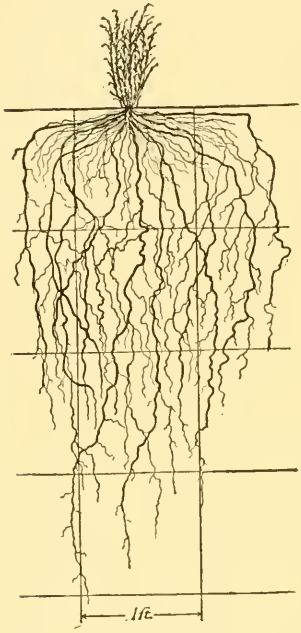

FIG. 75.—Root system of *Artemisia* (*after Weaver*).

Of the many common types of xerophytes or desert plants, the most peculiar are the cacti (Fig. 76). These plants have thick fleshy stems, and their leaves have lost their assimilative function and have turned into spines. Because of their shape, the cacti combine a small surface with a considerable volume. Usually they contain large amounts of water which is spent very slowly. Experiments have shown that a cactus can thrive for several months without an external water supply. The root system of this plant spreads near the surface of the soil. During a rain, which occurs in every desert, water is absorbed quickly and collected into the stem. While in the longer dry periods the small rootlets die and only the thicker roots remain and become covered with a corky layer. The epidermis covering the stem of the cacti has a very thick cuticle and only a few stomata which are almost always closed. They spend their water very scantily, therefore, even in the greatest heat. But the absorption of carbon dioxide is thus also checked and cacti are distinguished by slow growth.

Physiologically related to the cacti are other typical fleshy plants, in many of which water is stored in the leaves. Such are, for instance, the aloes and agaves of the tropics (Fig. 143), and species of *Sedum* (Fig. 77) and of *Sempervivum* of the temperate zone. Such plants grow either in sand or on rocks, stone walls, roofs, and in general where the thin soil layers often dry out. All such fleshy plants are called succulents.

Quite different from these are the thin-leaved xerophytes. To this type belong such plants as *Alhagi Camelorum*, the wild alfalfa of the steppes, some representatives of the *Cucurbitaceae*, as *Citrullus colocynthis*, while the species of sagebrush, *Artemisia*, found in semideserts, closely approach this group. These plants have thin tender leaves, which wilt rapidly when a branch is cut from the plant. The intensity of transpiration is very high in plants of this type, especially in sunlight. Even on very hot dry days they keep their stomata wide open, losing considerable amounts of water, but assimilating energetically. As a result of this vigorous transpiration, xerophytes of this type are able to lower considerably the temperature of their leaves. In the hot air and soil of the desert this protection against overheating is of great importance. The considerable loss of water is replaced from the lower layer of the soil, as xerophytes of this type possess an extremely well-developed root system. Where the water table is not too deep, the plants are able to reach it with their long roots and may stand, therefore, the greatest heat. Where water cannot be reached, the root system spreads over a very great volume of soil. During the driest

FIG. 76.—Gigantic cacti in an Arizona desert.

FIG. 77.—*Sedum acre.*

periods, however, water shortage commonly sets in. These plants are often in the state of permanent wilting, throwing off a part of their leaves and even branches.

The thin-leaved xerophytes are commonly covered by a dense layer of gray or whitish hair. This is illustrated by sagebrush and many other plants of dry plains. This hair has often been regarded as a means of checking transpiration. This, however, is an erroneous conception, as experiments have shown that the intensity of transpiration is very high in such plants. These hairs must be looked upon as forming a light filter to protect the plant against overheating and the chlorophyll apparatus against a too bright light. They also serve as a protective covering against the attacks of animals, as do the spines and the bitter, astringent, and pungent substances which the plants often possess. An important physiological peculiarity of xerophytes of this type is the high concentration of the cell sap, which enables the wilting plant to develop an enormous suction tension and to draw water from the soil very rapidly. Moreover, it is possible that the soluble substances which accumulate in large amounts in these plants make them more resistant to permanent wilting.

The third type of xerophytes are sclerophyllous plants with rigid leathery leaves. To this group belong many prairie grasses, such as *Stipa* and *Festuca*, some *Umbelliferae*, as *Eryngium*, etc. The sclerophyllous xerophytes are especially widely distributed in the dry districts of the Cape Province, Africa, where may be found many representatives of the family *Ericaceae*.

These plants are characterized by an unusual capacity to resist permanent wilting. The margins of the leaves commonly curl inwards and the stomata are hidden in a tubelike depression (Fig. 78). Thus, they are able to preserve for a long period the last portion of water indispensable to life. The physiology of these plants has been investigated very little. The sap concentration of the cells is very high in xerophytes of this type, while in the succulents it is, on the contrary, very low.

In addition to these groups of true xerophytes, which are able to withstand in one way or another the driest and hottest weather, there are found in the desert also plants known as pseudo-xerophytes. These are the so-called ephemeral plants, characterized by an extremely rapid development. Within the span of 3 or 4 weeks these plants will germinate, bloom, set fruits, produce

mature seeds, and die. Their whole life cycle occurs during the short moist period, usually in spring. During the remainder of the year, they exist in the form of ripe seeds that cannot be affected by desiccation. In all their other characteristics these plants are typical mesophytes.

The groups discussed by no means exhaust the various xerophytes. The types sharply delimited here, moreover, are connected by a series of plant forms showing imperceptible transitions from one group to another. A detailed examination of these types belongs to the field of ecology.

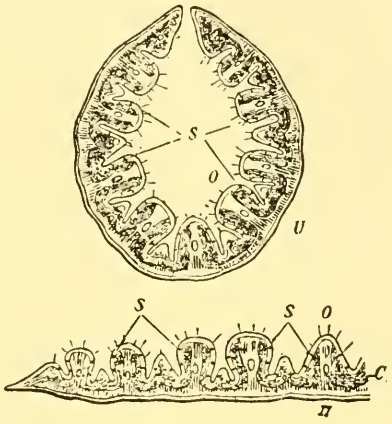

FIG. 78.—Leaf of *Stipa* curled into a tube.

57. Correlation between Assimilation and Transpiration. Efficiency of Transpiration and the Water Requirement.—The transpiration of plants is closely connected with assimilation by the mere fact that carbon dioxide enters through the same stomatal openings through which water vapor escapes. Moreover, the transpiration current carries with it mineral salts and nitrogen compounds necessary for the synthesis of organic substances. The correlation between these two processes is, therefore, very important not merely for the physiological characterization of the plants, but also for the evaluation of the external conditions under which the plant has to work.

This correlation, or the efficiency of transpiration, is usually expressed in grams of dry weight accumulated per liter of water lost. Under rather constant environmental conditions the value thus obtained remains quite stable for each plant. In central Europe, for instance, cereals like wheat have a transpiration efficiency of 3; those of the type of maize, about 5. The efficiency of transpiration will vary with changes in the external conditions. In the hot and dry climate of Tiflis (Caucasus) it is only 1.5 for wheat, and 2.5 for corn. If, on the contrary, a plant is moved to the moist atmosphere of a greenhouse, the efficiency of transpiration may be raised to 10 or even higher.

Instead of the efficiency of transpiration a converse value is often used in agricultural literature, that is, the number of grams of water spent in the production of 1 g. of dry weight, which is called the transpiration coefficient, or the water requirement. For the majority of crop plants this value is from 300 to 400 under the conditions of central Europe. It was believed for a time that the adaptability of a plant for growing in drouth regions may be judged from the transpiration coefficient. It seemed natural that the more xerophytic a plant is, the more efficiently it will spend water. The extensive investigations of Briggs and Shantz, in the United States, and of Maximov and Alexandrov in the Caucasus, have shown, however, that no direct correlation between drouth resistance of a plant and its water requirement can be established. In many typical xerophytes, for instance, the sagebrush, the efficiency of transpiration is very low, but its intensity is high.

58. Effects of Environment on Structure of Transpiring Organs. Xeromorphism and Drouth Resistance of Plants.— The transpiring organs of plants, which in practically all cases are the leaves, exhibit a considerable plasticity. Leaves of different plants and even of the same plant show considerable differences in structure, depending on the external environmental factors under which they develop.

That certain regularity in the structure of leaves depends on their position on the plant has been established by Zalensky. He has found that leaves on the upper half of a stem are always different from those below. The higher the position of a leaf, the smaller are the dimensions of its cells, but the greater the number of stomata per surface area, the size of each stoma being smaller, however. Terminal leaves have also a thicker network of vascular bundles, a greater number of hairs per surface area, and a thicker layer of palisade tissue. This dependence of structure on position has been called the Rule of Zalensky. Figure 79, showing the vascular network in an upper and a lower leaf of tobacco, may serve as illustration of this rule.

These anatomical peculiarities may be correlated with physiological differences. The upper leaves are distinguished by higher assimilation and more intense transpiration. The osmotic pressure of their cells is higher, and in wilting the upper leaves draw water from those lower down. When permanent wilting takes place, the lower leaves will release all their water and die. In

case of water shortage, stomata of the upper leaves remain open longer. As this is a characteristic feature of many xerophytes, this structure has been called "xeromorphism."

These differences in structure of the upper leaves may be due to the fact that they develop under the conditions of a somewhat lower water supply, which may result in smaller dimensions of their cells. The same xeromorphic structures may be induced by the direct influence of external factors on the plant as a whole, such as increased dryness of the air, reduced soil moisture, and periodical wilting. Plants developing under these conditions are smaller in size, but have a greater drouth resistance.

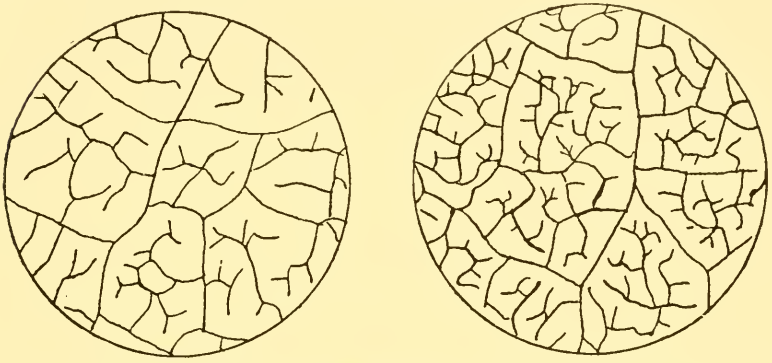

FIG. 79.—Vascular network in a lower (left) and an upper (right) tobacco leaf (*after Zalensky*).

Similar changes are produced by increased light intensity. Generally, sun leaves differ from shade leaves by the same characteristics as the upper leaves differ from the lower ones. Especially marked are the changes in the development of palisade parenchyma. Of the many physiological characteristics of sun leaves, an increased assimilation, at least in strong light, and an intensive transpiration are especially marked. Consequently, sun leaves may be considered as xeromorphic leaves.

The study of the anatomical and physiological peculiarities of xeromorphic structures is of great importance in the determination of differences in drouth resistance among varieties of crop plants. Recent investigations (Maximov, H. Walter) have shown that resistance to drouth is not due to a reduced expenditure of water, but to the capacity to endure wilting, which inevitably

accompanies drouth. These characteristics are closely connected with a xeromorphic structure. In selecting drouth-resistant varieties attention must be paid to the size of the cells and to other anatomical features, as has been pointed out long ago by Kolkunov. Naturally the point in question is not the small size of cells, but the physiological peculiarities connected with them.

CHAPTER VII

TRANSLOCATION OF WATER

59. The Path of Water Translocation.—In mosses and herbaceous plants, the organs absorbing water from the soil are separated from those releasing it to the atmosphere. In trees the distance between the absorbing and the transpiring organs may be several hundred feet. The water current, therefore, must move in the plant through a series of tissues, beginning with the root hairs, which absorb it from the soil, and ending with the cells of the leaf parenchyma which set free the water vapor in the intercellular spaces.

From both a physiological and a structural point of view the course of water movement in the plant may be divided into two parts. Water moves mainly through the vascular system of the plant, which consists of dead cells, such as the tracheids and vessels, representing as it were water-pipe lines. In herbaceous plants the distance of this path may be several centimeters, while in trees water may move in this manner hundreds of feet. The second part of the water-translocation system consists of living cells. Through these water moves for only short distances of a few millimeters, or fractions thereof. There are two small layers of tissues thus traversed by water; one in the root, from the surface of the root hair to the vessels in the central cylinder; the other in the leaf, from the vessels of the fibro-vascular bundles to the mesophyll cells bordering the intercellular spaces. The course taken by the water current through these tissues is represented diagrammatically in Fig. 80. On the right is shown a root hair transmitting water through a number of parenchyma root cells. Having passed the endoderm and pericycle, it enters the vessels of the central cylinder through which it is moved to the leaf. Here it passes again through a number of parenchyma cells and is finally evaporated into the atmosphere.

These few millimeters of translocation in the living cells repre-

sent greater difficulties than do the many meters traversed in the conductive tissues. The ease with which water may be translocated through the vessels and its slow movement from cell to cell may be seen from the following simple experiment. When a succulent, leafy stem with numerous parenchyma cells, for instance of the balsam plant, is cut and placed into water, it will remain fresh for a long time, as the water readily enters through the cut vessels and ascends to the leaves. When the cavities of the vessels are sealed up, however, by keeping for a time the cut end in liquid gelatine, then cleaning the surface and replacing the stem into water, the leaves and the tip of the stem will begin to wilt. This

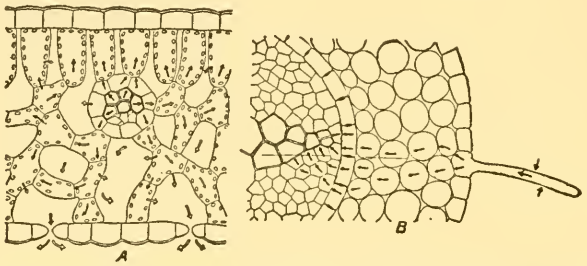

FIG. 80.—Diagram showing the course taken by water current in the plant. The black arrows show movement of water in liquid form; the light arrows, that of water vapor (*after Smith, et al.*).

shows the slow permeability to water of the parenchyma tissues of the cortex and pith.

The rapidity with which water will move in the vessels and in the parenchyma is dependent upon the method of transport. It flows through the vessels as through hollow tubes, obeying the general laws of hydrodynamics. In the parenchyma cells water is translocated osmotically and its movement meets with considerable resistance.

60. The Osmotic Translocation of Water.—Imagine a vertical series of cells of which the lowest dips in water, while the other cells are above its surface (Fig. 81). In order to simplify the scheme, assume that only the upper cell evaporates water, while the other cells are protected against water loss by impermeable lateral walls. When all of the cells are saturated with water, there will be no movement of this fluid. As soon as evaporation begins, the upper cell loses part of its water, the volume of the cell decreases, turgor

pressure becomes less, and, as a consequence, suction tension develops (Art. 42). The upper cell now begins to draw water from the one beneath it, which up to that moment was saturated with water and, therefore, showed no suction tension. The loss of water creates a suction tension in the second cell and it begins to draw water from the third, and so on, until the lower cell is reached. Then this cell begins to absorb water from the container, in which its lower part is immersed.

It must be remembered that the moving force that drives the water current from cell to cell is the difference in the suction tension and not in the absolute magnitude of the osmotic pressure. Let the osmotic pressure of the first cell be 10 atmospheres, for instance; and of the second, 20 atmospheres. As long as they are saturated with water they are in equilibrium with one another, as the surplus of pressure in the second cell is balanced by the greater tension of its walls. But, as soon as suction tension arises in the upper cell, it begins to draw water from the lower one, in spite of the fact that osmotic pressure is greater in the second cell. In order to make the water current, induced by evaporation in the upper cell, move through a series of cells, suction tension must be distributed in these cells in regularly decreasing order. In the upper cell, suction tension must be the greatest; in the following cell it must be less; in the next, still less, etc. The least suction tension must be in the lowest cell which absorbs water from the surrounding medium.

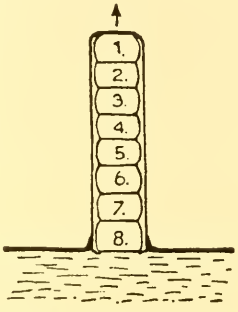

FIG. 81.— Diagram illustrating translocation of water in leaf parenchyma.

The difference in suction tension of two neighboring cells is determined by the resistance met by the water current in passing from one cell into the other. This resistance, in its turn, depends on the rate of the water current. It increases as the rate of the water current augments. Ursprung was able to trace the increase of suction tension in the cells of the palisade parenchyma of the ivy leaf, in proportion as these cells were farther from the large fibrovascular bundles supplying them with water. In the third cell from the bundle, he found a suction tension equaling 12.1 atmospheres; in the two hundred and tenth cell, 32.6 atmospheres. In

order to traverse 207 cells, a total difference in suction tension of 20.5 atmospheres was required, or about 0.1 atmosphere per cell. Although in other cases somewhat different figures were obtained, still they were close to this value.

The strong resistance met by water in moving from cell to cell shows that long distances cannot be covered in this way; hence, the other means of translocation of water through dead, empty, elongated cells with numerous pores, the tracheids, or still better through hollow tubes with very thin septa, the tracheae or vessels. Lower plants, such as the lichens and mosses, have no true vascular systems. They, therefore, are small in size. The development of tracheids in ferns was an important step forward in the evolution of plants.

61. Translocation of Water in the Vessels. Dynamics of the Water Current. The Influence of Transpiration.—The vessels and tracheids, being mere tubes filled with water, possess neither suction nor any other power that may cause the movement of water. Because of the very small dimensions of those elements, capillarity might be considered as the only element developing considerable force. Confronted by the problem of the upward movement of water through the vessels, early investigators, therefore, tried to explain this phenomenon by capillarity. The source of force causing the capillary rise of fluids in narrow tubes is the force of adhesion shown by the concavity of the upper meniscus. But the plant vessels are completely filled with water. No menisci are found in them. Moreover, if even such menisci existed, with an average diameter of the vessels = 0.1 mm., water would be able to rise on the strength of capillarity only about 30 cm. In trees, the water has to be lifted many meters. Hence the theory explaining the upward movement of water in the plant by capillarity was soon discarded.

The water-conducting tissues being passive elements, the active movers of the water current are the living cells which adjoin the vascular system in its upper and lower end. At the base of the plant, this work is performed by the cells of the root parenchyma which are in close contact with the vessels, while above, it is accomplished by the cells of the leaf parenchyma, which clasp the tips of vascular bundles (Fig. 82, also details in Fig. 80). As a result of the activity of these cells, the water current moves through the dead tissues of the wood.

The root parenchyma cells are able to pump water into the vessels with a force of 2 to 3 atmospheres. Hence, with feeble transpiration the surplus of water may be forced out of a plant in the form of drops. The importance of this lower motive force, however, is not nearly so significant as is that of the upper terminal motor, the transpiring cells of the leaf parenchyma.

That transpiration is a sufficient force to convey the water supply of the plant may be seen from the well-known fact that portions of a plant, when cut off and placed in water, preserve, under favorable conditions, their freshness for a long time, using up a considerable amount of water. The mechanism of the transpiration pull is not so very complicated after all. It is based on the fact already discussed that every cell, not fully saturated with water, shows a suction tension of several atmospheres; hence, when brought into contact with water, it absorbs water with a proportional force. The parenchyma cells of a leaf, for example, will absorb water from a vessel with the force of several atmospheres. This avidity for water will be the greater, the more the cell loses it, or the stronger the process of transpiration. The upper terminal system thus represents a self-regulating mechanism which absorbs water in proportion to the rapidity with which it is spent.

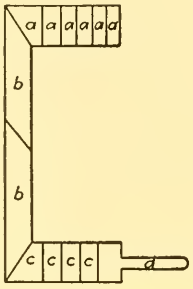

FIG. 82.—Diagram showing the arrangement of the terminal mechanisms of the water current: leaf and root parenchyma and vessels connecting them: (*a*) leaf parenchyma; (*b*) vessels; (*c*) root parenchyma; (*d*) root hair (*adapted from Walter*).

The suction of water by the transpiring leaves can be readily measured. A leafy shoot may be conveniently used for this purpose. It is inserted into the upper end of a long tube filled with water, the lower end of which dips in mercury (Fig. 83). After a short time it will be seen that as the water filling the tube is used up, it will be replaced by mercury, which may rise to a considerable height. The demonstration is brought to an end not by the ceasing of transpiration but by an accessory phenomenon. Air from the intercellular spaces of the shoot will begin to enter the tube, finally filling its whole upper end and breaking the connection between the vessels of the plant and the water.

This entry of the air into the tube interferes with the exact determination of suction tension of the leaves as measured by the

rise of the mercury. Indirect measurements, based on the determination of suction tension in separate cells of a leaf, have shown, however, that a force of several atmospheres is attained. Thus, in the plant at the lower and the upper ends of the vascular system are two forceful pumps, the one below lifting water, the other pulling it. These two mechanisms provide the plant with a con-

Fig. 83.—Transpiration pull shown by rise of mercury in the tube (*after Molisch*).

tinuous water current. The upper terminal device shows a number of advantages over the lower one. It is able to develop a much greater force, it is self-regulating, and it works at the expense of radiant energy, which heats the leaf and increases transpiration. The lower mechanism works at the expense of nutritive substances supplied by the leaves. It is little wonder, therefore, that the upper equipment is more efficient than the lower, and that most of

the water used by the plant is supplied by its action. Only in early spring, when the leaves have not yet expanded, or in case of a very moist atmosphere, when transpiration falls to a minimum, is the principal rôle in raising water assumed by the root cells.

62. The Rise of Water in the Trunk of a Tree. Water Tension in the Vessels. The Theory of Cohesion.—The action of the upper and lower terminal mechanisms readily explains the rise of water of several centimeters and even of several meters in the stems of herbaceous plants, shrubs, and even small trees. In extending this explanation to the lifting of water in large trees to a height of 100 ft. or more above the soil surface, difficulties are met with which will now be discussed.

In the first place, root pressure is too feeble a force to drive water with sufficient rapidity to a considerable height. Only in the spring, when root pressure is especially high and the loss of water by the undeveloped leaves is very low, it may be pumped with such force by the roots that the sap will exude from wounds of the trunk and cut shoots. As soon as the leaves are completely unfolded, the force of transpiration will be so much greater than that of root pressure that all injured places will absorb water. This circumstance permits the introduction of solutions into the stem of plants, mentioned in Art. 48. The surface of a cut stump usually does not exude water in summer, but on the contrary absorbs it.

Although, in the trunk of a tree the suction tension of the leaves is transmitted downwards to a considerable distance, it is known, however, that a suction pump is not able to lift water to a greater height than about 30 ft. At this height the weight of the water column is equal to the pressure of 1 atmosphere. No rarefaction of the air will induce water to rise above this level.

This, however, is only an apparent contradiction. The lifting of water by a pump and the sucking of water by the cells are quite different phenomena. The drawing of water by a pump is induced by a decrease in density of the air in the cylinder situated above the water surface. The atmospheric pressure exerted on the water in the container into which the pump is inserted will cause the water to rise to a height corresponding to the lowering of air pressure in the cylinder. It will be understood, therefore, that only a maximal rise corresponding to 1 atmosphere, or 10 m. of a water column, can be attained.

Water suction by the cells, on the other hand, proceeds quite differently. Here the motive power is the difference between the osmotic suction of the cell sap and the counterpressure of the elastically distended cell walls, which may attain a magnitude of several atmospheres (Art. 42). This force permits the cells to suck water from the adjoining vessel. The pull is then transmitted to the whole water column in the stem, since the water absorbed by the cell immediately draws the rest adjoining it. The air or atmospheric pressure plays no part whatever in the whole process.

The force that makes the water molecules follow each other is called cohesion. It is the same force that holds an iron bar together, permitting it to be lifted by one end. The force of cohesion in solid bodies may be determined in a comparatively simple manner owing to the low mobility of their molecules and their constant external form. When the tensile strength of a bar of metal is tested, the strain at which it breaks is considered as being equal to the force of cohesion. Thus a steel bar, for instance, breaks at a strain of about 80 kg. per square millimeter, which approximately corresponds to the tension of 8,000 atmospheres.

Since liquids have molecules in a more mobile state, tests of the strength of their cohesion are subject to greater difficulties and errors. The determination has to be conducted in a tube with two very closely fitting pistons. It has been proven by means of proper equipment that the cohesion force of water molecules and their adhesion to the surface of the pistons is very high indeed. Only by applying to the pistons a pulling force of many atmospheres can the water layer between the pistons be ruptured. Still it is difficult to establish whether the water column itself has been ruptured or whether it has been only torn away from the surface of the piston or the tube. The latter seems more probable, since on the surface of glass or metal there is always a thin adsorbed air layer, which prevents the perfect adhesion of water and probably induces premature tearing.

That the cohesion power of a liquid permits it to rise to a height exceeding that induced by barometric pressure was proven by Askenasy's (1895) well-known experiment. He took a long glass tube to whose upper end a funnel containing hardened plaster of Paris was sealed. The tube was filled with boiled water and the plaster block filling the funnel was soaked with it. The lower end of the tube was dipped in mercury. Care was taken that no air

bubbles were left anywhere (Fig. 84). The mercury in the tube began to rise after a short time, owing to the evaporation of water from the surface of the plaster and its drawing upon the supply in the glass tube. If all the air was removed from the apparatus and the pores of the plaster block were sufficiently small to prevent the entry of air from outside, then the mercury rose finally to a height somewhat exceeding that caused by barometric pressure, as a result of the cohesion of the water molecules and their adhesion to those of mercury.

This experiment illustrates the rôle played by cohesion, and the influence of water evaporation from the surface of a moist porous body. The force of adhesion demonstrated by the concavity of the menisci in the capillaries of the plaster of Paris is the active force in this case. The greater the resistance to a pull, the more concave will be the water menisci and the stronger the tension on them. Transpiration pull evidently is based on this mechanism of capillary tension. The cell walls, from the surface of which evaporation takes place, are made up of cellulose in a colloidal state. They have microcapillaries with their micromenisci of water. When the resistance to water flow is increased, these menisci are drawn into the tiny capillaries, they increase in concavity and as a consequence suction tension is augmented. Therefore, they draw water from the cell, inducing in it a suction tension of corresponding strength and osmotic in nature. This pressure in its turn induces the suction of water by the cell from the vessels of the plant.

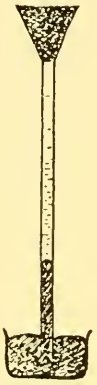

FIG. 84. — Suction produced by evaporation of water from plaster of Paris (*after Walter*).

To approach the conditions found in a plant, Ursprung used, instead of a glass tube, the stem of a liana. He obtained a rise of mercury of twice the barometric height, since the walls of the vessels in a plant stem are not only moistened but also imbibed with water.

These experiments still do not give a true conception of the real magnitude of cohesion force that retains the water molecules near one another and prevents the rupture of the water column in the vessels of a plant. The cohesion power of water cannot be expressed by a few atmospheres. The simultaneous experiments of

Renner and of Ursprung (1915) have shown that it may be as high as 300 to 350 atmospheres. These experiments were conducted on the so-called annuli of the sporangia of ferns (Fig. 85). These annuli are made up of dead cells, whose inner lateral walls are thickened, while the outer walls are thin. In cross-section such a cell looks like a horseshoe, the ends of which are connected by a thin wire. When the sporangium matures, these cells, originally filled with water, dry up. The volume of water in them decreases and the cells gradually shrink, drawing the thin walls inward, so that the ends of the thick walls approach each other (Fig. 86). Thus a highly strained spring is obtained, tending to rupture the water within the cells, or at least to tear it from the walls. The water

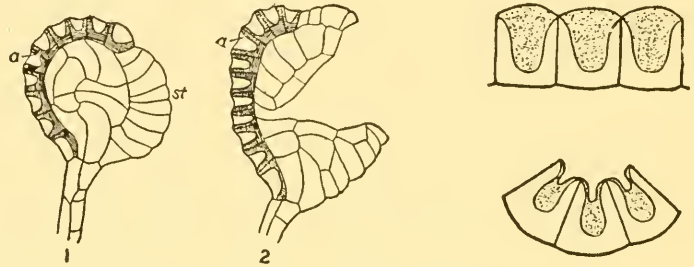

FIG. 85.—Sporangium of a fern; (1) with curved, (2) with straightened annulus.
FIG. 86.—Cells of the annulus of a fern sporangium; above, saturated with water; below, shrunken as a result of desiccation (*after Walter*).

column finally does rupture, producing in the cell a Torricellian vacuum. The strained springs straighten at once, the whole annulus rapidly contracts, scattering spores as from a catapult. The whole process may be readily observed under a microscope. The force necessary for such a compression of the cell walls in the annulus was determined by immersing them in highly concentrated solutions of various substances. It proved to be of the magnitude of 350 atmospheres.

This immense force of cohesion of the molecules is more than sufficient to raise water to the top of the highest tree, as the pressure of a water column 100 m. high is equal to but 10 atmospheres. Hence the ascent of water in a tree may be represented in the following way according to Dixon: Near the top is the evaporating leaf parenchyma in whose cells develops a suction tension of many atmospheres. These cells draw water from the vessels of

the vascular bundles, which pervade the whole leaf. Consequently, a pull on the water in these vessels is produced, which is transmitted to the vessels of the stem and the roots. The water in the wood appears to be suspended to the cells of the leaf parenchyma, but in the root tips are also parenchyma cells which, with a sufficient amount of water in the soil and with a slow utilization, support, as it were, the water threads suspended from the leaf cells. Thus the strain on the leaf parenchyma is reduced and the water threads are even pushed upward with considerable force. With lack of water in the soil and with a simultaneous intensive transpiration, as, for instance, on hot summer days, the supply of water by the root cells cannot keep up with the loss of water from the leaves; in this case the influence of suction of the leaves is transmitted through the highly strained threads in the vessels, even as far as the root cells.

63. Compression of Vessels during the Ascent of Water. The Presence of Air in Vessels.—The water threads filling the vessels tend to increase in length and to decrease in diameter, similar to a stretched rubber tube. This tendency would soon cause their breaking into separate drops, which is actually observed when water falls in a thin jet, if they were not contained within the walls of vessels to which they are closely attached by the force of cohesion. When water is under a very high strain in the vessels, the walls of the vessels will be drawn inward and the diameter will decrease. This transverse compression of the vessels is counteracted by the annular or spiral thickenings, which keep the walls apart. On the whole, the compression is rather small. It may be perceived under the microscope by observing the vessels of plants that have a sufficiently transparent stem, for instance young plants of pumpkin or balsam, during rapid wilting, when the suction tension of the leaves attains its maximum, while the roots are unable to supply water from the dry soil. This compression of vessels may be observed also on a whole tree trunk by means of the dendrograph. This is an extremely sensitive apparatus, devised by MacDougal, which records slight changes in the thickness of the trunk (Fig. 87). This apparatus shows that during the afternoon hours of bright days, a contraction of the trunk takes place, followed by an expansion at night (Fig. 88).

The elastic compression of the walls of vessels during periods of increased transpiration is the cause of a phenomenon which has

been known for a long time, but usually misinterpreted. If on a hot day the stem of a rapidly transpiring plant is cut, the air will quickly enter the vessels. When placed in water such a stem will soon wilt, as the air clogs the vessels, preventing water from rising. If the stem is cut under mercury, the latter will enter forcefully into the vessels and rise to a considerable height. In order to keep plants fresh, cutting must be made under water.

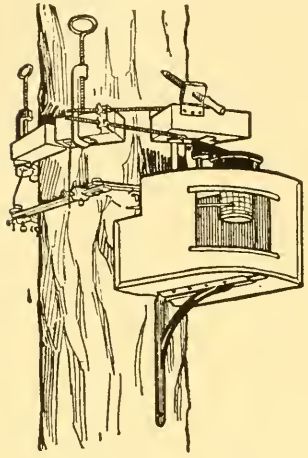

FIG. 87.—MacDougal's dendrograph.

The reason for this phenomenon is that the vessels of the plant, being compressed with the force of several atmospheres, abruptly widen when the strained water threads are cut. Air, mercury, or water, depending on the medium used, will rush into the empty space. Yet for a long time this phenomenon was explained in a different way. It was supposed, that besides water, the vessels contain highly rarefied air in the form of small bubbles. When a cut was made this air contracted and drew after it mercury or water. The recent investigations by Renner and his coworkers have shown, however, that vessels which conduct water normally do not contain air, but are filled with water throughout their length. Air will enter only as a result of a mechanical injury. If

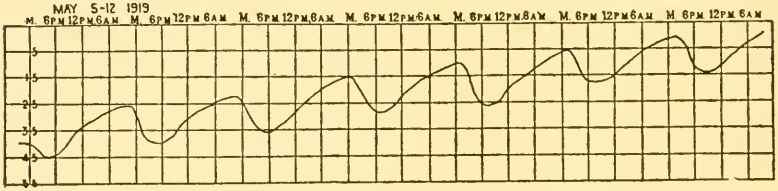

FIG. 88.—Curves showing diurnal fluctuations in the thickness of a tree trunk, recorded by the dendrograph (*after MacDougal*).

cutting is done carefully under mercury, the meniscus of the latter will adjoin the water, no air bubbles being present in the vessels. The supposition of the presence of air bubbles in the vessels has prevented for a long time the acceptance of the cohesion theory,

as these bubbles would interfere with the translocation of water and would, moreover, rupture water threads at such high tensions as are observed in the trunks of trees. It is known now that air is present only in the older wood, which has ceased to function for water conduction. The younger parts, through which water moves, contain no air. Air that enters the vessels as a result of mechanical injury, for instance in wounding, is not able to move farther than through the first septum. Such injuries will cause the exclusion of only small portions of the general system of water supply (Fig. 89). The presence of cross-septa in the vessels, which show of course a considerable resistance to the translocation of water in the wood, is an indispensable condition for the regular functioning of the whole water-conducting system. The air which clogs the vessels is frequently dissolved again and the elements having temporarily suspended their functions begin once more to take part in the conduction of water.

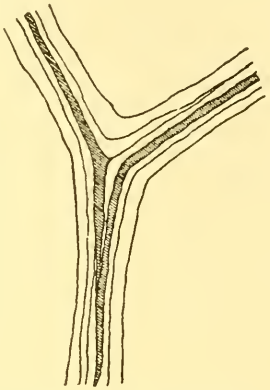

FIG. 89.—Vascular bundle of a wilting leaf, into which air has entered in cutting. Vessels filled with air cross-hatched; vessels filled with water in outline (*after Bode*).

64. The Rate of Water Movement in Plants. Resistance of Wood. Participation of Intermediate Factors in Water Transportation.—As wood consists of exceedingly narrow tubes, which moreover are provided with septa, it must show a strong resistance to the ascending water. Naturally, this resistance becomes an additional encumbrance to the cells of the leaf parenchyma.

This additional resistance was formerly rated very high, considering the pressure necessary to force water through septa in the wood. The comparatively low rate at which the water moved in the plant, however, was not taken into consideration. Exact determinations by Ursprung have shown that with increase in height in the trunk, the suction tension of the parenchyma cells adjoining the vessels increases. But this increase constitutes only 0.3 to 0.4 atmosphere per meter in height. This is a small value indeed when compared with the increase in tension of water moving through the parenchyma. In this movement from cell to cell, the suction force increases by 0.1 atmosphere. If the average dimen-

sions of these cells are assumed as 0.1 mm. (they are really one-third to one-half as large), it will be found that the translocation of water in the parenchyma for a distance of 1 m. would require the immense force of 1,000 atmospheres. This shows the value of special conductive tissues for dry-land plants of large size. They increase the movement of water 2,500 to 3,000 times.

The actual translocation rate of the water through wood is not high. According to the calculations of Farmer, for deciduous trees, it is, on an average, 20 cc. per hour per square centimeter of cross-section of wood, and for conifers but 5 cc. If these values are compared with the rate at which water moves through ordinary city mains, which often reach 100 cc. per square centimeter of cross-section per second, or with the rate at which the blood is conveyed through the arteries, normally 40 to 50 cc. per second, it is found that water moves through ordinary water pipes several thousand times more rapidly than through a plant. With such slow translocation of water, the resistance to filtration cannot be very great. The suction pressure developed by the cells of the leaf parenchyma seems to be quite sufficient not only to keep in suspension the whole mass filling the wood, but also to draw it upward.

When the diameter of the vessels is increased and the number of septa decreased, the resistance of the wood to the translocation of water is considerably lowered. Such increased conductivity is found in lianas, the twining and creeping plants of tropical forests. The individual vessels in their stems may be 2 m. long and 1 mm. in diameter. These are vessels of exceptionally large size, however. In the majority of deciduous trees the vessels usually do not exceed 10 cm. in length and 0.5 mm. in diameter. Such smaller dimensions though reducing conductivity, secure certain other advantages to the plant. The small diameter diminishes the danger of breaking of the water threads, while the septa prevent the air, which for one reason or another may have entered, from spreading through the whole system.

Thus it is seen that in the presence of a cohesion force, the work of the upper and the lower terminal mechanisms is quite sufficient to lift water to the crown of a tree. The main containers of the water stream remain as passive as the iron pipes of a water-system. Formerly, however, the cohesion theory was not elaborated sufficiently, and the resistance of the wood to filtration and

the rôle of the air entering separate vessels were considerably overrated. Frequently, for this reason, the idea of the insufficiency of the terminal forces presented itself and the necessity was felt of looking for some intermediate mechanism which might support the terminal ones. Most frequently the living cells of the wood were regarded as such an intermediate mechanism, especially the cells of the medullary rays and those of the wood parenchyma, which adjoin the vessels. It was supposed that these cells have the same faculty as the cells of the root parenchyma, of pressing water through them in one direction only. Lately this view has been strongly supported by the Indian scientist Bose. This author tries to explain not only the translocation of water in the stem, but also the loss of water in transpiration, by the pulsation of the living cells. He even contends that the water moves not through the wood but through the bark, where the living cells are much more numerous. That water is indeed able to move only in wood layers that are still alive seems to favor the idea of the participation of the living cells. In the dead inner layers the vessels are filled either with resinous substances, forming the so-called heartwood of the tree, or simply with air. They no longer participate in water conduction.

This theory of intermediate mechanisms has been refuted through a series of experiments. Votchall (1897) has proved by his comprehensive work that in a living tree trunk, placed horizontally, water moves from the lower end to the upper one at the same rate as from the upper to the lower. This clearly shows the absence of any valves in the wood, preventing the downward movement of the water. But without such valves the supposed intermediate mechanism would not be able to work. Moreover, Strasburger (1893) and other investigators have shown that when poisonous solutions, such as picric acid, are introduced through cut vessels, they will rise unchecked in the trunk to a height of several meters, or clear to the upper leaves, though they certainly poison all living cells on their way. Likewise, the unchecked rise of water through portions of a herbaceous stem, killed by heat, freezing, or toxins, has been observed.

After some period, however, such killed portions of a stem cease to conduct water and their vessels become filled with air. The exact reason for this behavior has not been established as yet. Some investigators suppose that it results from changes occurring

in the properties of the cell walls under the influence of heat or of chemical substances. Others are inclined to think that the living cells of the parenchyma, without directly participating in the process of raising water, exert an indirect influence by absorbing the air that appears in the vessels. This question requires still further study.

Part III

UTILIZATION OF RESERVE PRODUCTS AND LIBERATION OF ENERGY

CHAPTER VIII

ORGANIC SUBSTANCES AS PLANT FOOD

65. Storage of Substances in Seeds and Other Organs. Their Utilization in Germination. Major Groups of Reserve Substances.—The process of nutrition of the plant may be divided into two stages. The first stage consists of the acquisition from the surrounding environment, the soil and the atmosphere, of the necessary raw materials, such as carbon dioxide, water, nitrogenous compounds and the various ash constituents, and the formation from these substances of the food products proper, mainly carbohydrates, fats, and proteins. This stage represents a specific characteristic of the plant organism. A synthetic activity of this nature, effected largely through the chloroplasts, is not available to the animal organism.

The second stage consists of nutrition in the narrow sense of the word, or the transformation of the synthesized products into substances from which the cells and tissues are formed. In the first place, this consists in the building of new masses of living protoplasm, and the construction of the secondary structures of the cell, its wall and various kinds of inclusions, etc. In the process of nutrition, the products manufactured by the leaf are subject to complete transformation. A considerable part of them disappear from the organism, being consumed in respiration and serving as material for the acquisition of the necessary energy. This phase of the nutrition of plants does not differ essentially from the nutrition of animal organisms. The fundamental difference between the nutrition of animals and plants is that plants consume substances made by their own synthesis, while animals feed on products formed by plants.

The first stage in the nutrition of plants has been discussed in the first part of the book. The most convenient way to study the second stage is by starting with the nutrition of the seed just coming to active life. Seeds, in the first place, contain comparatively large and conveniently observable reserves of nutritive substances and, secondly, the utilization of food products proceeds in them at a

very high rate, while the process of organic synthesis has not yet begun. Other convenient objects of such a study are the various organs containing large food reserves, which serve in part for their conservation during the winter or through periods unfavorable to synthetic activity or for vegetative reproduction. Good examples of such structures are the fleshy root stocks, tubers, bulbs, and storage roots, or the storage tissues of tree trunks. Although the reserve substances stored by plants in the various organs are quite varied in their chemical nature, three major groups, the carbohydrates, fats and proteins, usually predominate. The first two of these substances are of a simple composition. Their molecules contain only three elements; carbon, hydrogen, and oxygen. They are, therefore, called the "non-nitrogenous compounds." The composition of the protein molecule, however, is more complex. Besides the three mentioned elements we find in it nitrogen, sulphur, and often phosphorus. In addition to carbohydrates, fats, and proteins, storage organs frequently contain various glucosides, phosphatides, alkaloids, organic acids, and other substances. Their rôle, though very little understood, appears to be of a secondary character. Attention, therefore, will be centered here upon the examination of the transformation of the major groups, referring those interested in the minor substances to more detailed texts of plant physiology or to special books on biochemistry.

In the seeds of various plants are found different quantitative proportions of the three major groups of reserve substances. Of the non-nitrogenous compounds, the fats and carbohydrates may replace each other in various plants. In the seeds of some, fats predominate, in others carbohydrates, primarily starch. It is customary, therefore, to divide them into two groups, the oily and the starchy seeds. In the former the fat content reaches 40 to 60 per cent, as the following table shows:

	Fat, Per Cent	Carbohydrates, Per Cent
Hemp	33	21
Flax	34	23
Sunflower	53	11
Poppy	41	23
Almond	53	19
Cocoanut	67	12

In starchy seeds, on the contrary, there is usually very little fat, as may be seen from these figures:

	Fat, Per Cent	Carbohydrates, Per Cent
Wheat	1.8	69
Oats	5.3	60
Buckwheat	2.7	72
Pea	1.9	53
Corn	5.8	66
Rice	1.3	77

In his agricultural activity, man cultivates primarily plants with starchy seeds. These give him cereals, which form the major part of his diet. In general, however, plants with oily seeds predominate in nature, being produced by 90 per cent of plants on earth. As they contain less oxygen and have a high energy value, of which details will be given later on, oils form more compact reserves of nutritive substances than carbohydrates.

Protein compounds always form a smaller part of a seed's reserves, rarely reaching 25 per cent of its total weight. Besides, oily seeds usually contain a larger percentage of proteins than starchy seeds. Hemp seeds contain 18 per cent of proteins; flax, 23 per cent; poppy, 19 per cent; almond, 22 per cent; nuts, 17 per cent; and sunflower seeds, up to 30 per cent, whereas in wheat there is but 12 per cent; in buckwheat, 10 per cent; corn, 10 per cent; and rice, 7 per cent. The starchy seeds of leguminous plants, however, are very rich in proteins, very much like the oil-containing seeds. Thus, peas contain 29 per cent protein; kidney beans, 23 per cent; soybeans, 34 per cent; and lupines as much as 40 per cent. This high protein content of leguminous seeds is undoubtedly connected with their ability to fix atmospheric nitrogen owing to the activity of the tubercle bacteria. Having at their disposal during the course of their lives an unlimited supply of nitrogen, leguminous plants supply their progeny with a liberal amount of nitrogenous substances.

The composition of reserve substances in the seeds of different types of plants is not constant. It is subject to considerable fluctuations, depending upon external conditions. Especially strik-

ingly does external environment affect the proteins in seeds. The drier the climate, the higher is the protein content in wheat grains. According to investigations by the Institute of Applied Botany in Leningrad, the same wheat, when grown in the Province of Smolensk (western Russia), gave grain with 10.9 per cent protein; in the Province of Kostroma (central Russia), 13.6 per cent; and in eastern Siberia, 18.2 per cent. Similarly, the nature of the soil exerts an influence upon the composition of the grain. Soils somewhat alkaline and with a low water-supplying power will likewise increase the protein content of the grain. The dry climate and the alkalinity of the soil of the southeast of Russia, therefore, while making harvests uncertain in this region, still impart to wheat a very high quality. The quantitative and the qualitative composition of fats in oily plants likewise depend upon climate. A dry and hot climate as a rule, makes seeds of the sunflower more oily and increases the relative quantity of the unsaturated fatty acids in the oil obtained from them.

66. Types of Reserve Carbohydrates. Their Products of Hydrolysis.—Of the many groups of carbohydrates, seeds contain mostly starch, a polysaccharide insoluble in water and of the empirical formula $C_6H_{10}O_5$. During the maturation of seeds, starch is formed in the leucoplasts of the reserve parenchyma, composing the main mass of the endosperm or of the cotyledons. It is present in the form of particles or grains of various shapes and sizes, each characteristic of certain species of plants. Hence, under the microscope, it is possible to determine from the structure of the grains to what kind of plants the starch may belong. This is one of the most important methods of recognizing and distinguishing admixtures in flour. In their detailed structure, starch grains are sphaerocrystals, composed of very fine, closely associated and radially arranged crystalline needles.

The formula $C_6H_{10}O_5$ does not express the exact composition of starch. All the methods of determining the molecular weight have disclosed that the starch molecule is very large, and that the formula should be written $(C_6H_{10}O_5)_n$. The cryoscopic method of determination has given an especially large size to the molecule. Various authors have suggested different values for n ranging from 30 to 638.

Being a polysaccharide, starch readily changes into sugar. Upon the action of dilute acids it produces glucose. The starch

molecule, therefore, may be considered as consisting of a number of glucose molecules, connected with each other through the removal of water. Accordingly, the formation of starch from glucose may be given by the following general formula:

$$n C_6H_{12}O_6 — (n - 1)H_2O \rightarrow (C_6H_{10}O_5)_n \cdot H_2O.$$

This formula shows that a starch particle contains 1 molecule of H_2O more than is expressed in the usual formula $(C_6H_{10}O_5)_n$.

The conversion of starch into sugar may be expressed:

$$(C_6H_{10}O_5)_n \cdot H_2O + (n - 1)H_2O \rightarrow n C_6H_{12}O_6.$$

Detailed investigations have shown that in the hydrolysis of starch the formation of glucose is preceded first by the formation of polysaccharides soluble in water, the dextrines, and then by the disaccharide maltose. Conversely, in the formation of the starch, the glucose molecules first unite in pairs, giving maltose, and then the maltose molecules are polymerized. The exact character of these intermaltose unions is not known. More recent investigations of Karrer and others make it seem highly probable that starch is built up of ring compounds, anhydrides of sugars, in which each ring, a diamylose, represents an anhydride of maltose. These rings, linked to each other by means of side valences, are thus polymerized into the starch molecule. The number of such rings in a molecule of starch, however, is not as large as was formerly supposed, probably only three or four. The enormous molecular weight of starch obtained by cryoscopic determinations is due to its heating in water, which gives colloidal solutions. This results in the formation of rather large and very stable aggregates, which upon the action of hydrolyzing agents fall apart, as a rule, only at the time of the rupture of the valences, which hold together the rings in the molecule. The actual molecular weight of starch may be represented therefore by the formula $(C_6H_{10}O_5)_6$ or $(C_6H_{10}O_5)_8$.

Starch is not soluble in cold water, but it swells considerably in it, absorbing as much as 40 per cent of water. This water is retained with a great force. Even in an air-dry condition starch will hold as much as 15 per cent water. Starch has a high specific gravity (1.5 to 1.6). It precipitates easily and hence may be sep-

arated by settling, which is made use of in its purification. The starch grain seems to have a double structure. The basic mass of the grain, called "α-amylose" is more soluble upon heating in water and is easily hydrolyzed. The other part, called "β-amylose" is less soluble. It remains in the form of a fine skeleton when the α-amylose fraction is dissolved. Both give the iodine reaction typical for starch, the α-amylose, however, showing more of a blue, the β-amylose a violet shade.

Cellulose is a carbohydrate closely related to starch. It forms the basic substance of cell walls and has the empirical formula $C_6H_{10}O_5$. Cellulose differs from starch in being considerably more stable. It is not soluble in hot water and is hydrolyzed with difficulty by dilute acids. The end product of the hydrolysis of cellulose, as in the case of starch, is glucose. The intermediate product is likewise a disaccharide but, instead of maltose, it is cellobiose. It is possible that the structure of cellulose is similar to that of starch.

Owing to its chemical stability, cellulose forms the basic skeleton of cell membranes. In reality, therefore, it is not a reserve carbohydrate. Analogous to starch are the hemicelluloses, also known as reserve celluloses. Hemicelluloses are found in the endosperm or in the cotyledons of many plants as thickenings of the cell walls, thus giving to the tissues considerable solidity.[1] Such, for example, is the horny endosperm of some palms (*Phytelephas* and *Coelococcus*), so-called vegetable ivory, from which buttons are manufactured. Of a similar structure are the stones of dates and the seeds of coffee, peonies, lupine, nasturtium, and other plants. The chemical composition of reserve cellulose is not fully known. Apparently it is not the same in different plants. Among the products of hydrolysis are usually found glucose, maltose, and arabinose.[2] Hydrolysis as a rule proceeds very easily, often faster than that of starch; hence, at the germination of the seeds rich in reserve celluloses, they dissolve very rapidly.

In the underground storage organs, which differ from seeds by having a high water content, the reserve carbohydrate most frequently is starch, with which the cells are sometimes literally gorged, as, for instance, in the potato tuber. However, other

[1] It is present often in lignified and non-lignified tissues in various vegetative organs.

[2] Also galactose and xylose.

reserves of soluble carbohydrates are often found in such organs. Inulin, for example, is found in the tubers of the Jerusalem artichoke (*Helianthus tuberosus*), in the dahlia, and in the roots of chicory, dandelion and other Compositæ. Upon hydrolysis it gives fructose. Of the disaccharides, sucrose or cane sugar is most often present. In some plants it accumulates in great quantities. Thus, the roots of the sugar beet may contain 20 to 25 per cent sucrose, the fleshy stalks of sugar cane about the same amount, and carrots close to 7 per cent. Sometimes monosaccharides may serve as storage products. Glucose, for example, accumulates in the bulbs of many plants, including the garden onion and its relatives. Sugars are likewise found in seeds. Sugar corn and chestnuts may contain as much as 11 per cent of cane sugar, and quite a considerable sugar content is present in the pistachio, almond, and other nuts, which make these sweet to the taste.

Glycogen, a widely distributed sugar among animals, is not found in higher plants, but it is very common in fungi. Yeast cells may contain 30 per cent of glycogen on a dry-weight basis. Chemically, glycogen is closely related to starch. The only product of its hydrolysis is glucose. Apparently, it differs from starch only by being in a higher degree of dispersion, upon which depends its greater solubility in water.

Closely allied to the poly- and disaccharides are the glucosides, compounds containing sugar with alcohols of the non-carbohydrate type, most frequently those of the aromatic series. The glucosides represent a large group of substances of wide distribution in the vegetable world. They are found often in the food-storage organs. Hence they may be regarded as special kinds of reserve substances, which at germination liberate the combined sugar. Amygdalin is one of the glucosides most prevalent in plants, being found in the seeds of the almond, peach, apricot, plum, apple, and other *Rosaceae*. It contains glucose, benzoic aldehyde, and prussic acid. The latter splits off very readily and imparts to these seeds a bitter taste. In the bark of various plants is found arbutin,[1] which is composed of glucose and hydroquinone, while the roots of others contain saponins, poisonous glucosides, the nature of which is not yet fully understood. When combined with water they give highly foaming solutions, useful in medicine

[1] Some *Rosaceae* contain phloridzin, which is composed of glucose and phloretin.

and industry (example, soap root). The toxicity and the sharp taste of many glucosides indicate that they are special protective substances which save the seeds and other parts of plants from being eaten or injured by animals. There are α-glucosides, *right-rotating* and split by the enzymes of yeast cells, and β-glucosides, *left-rotating* and split by emulsin, which has no effect whatever on α-glucosides. The difference between these two types of glucosides is explained by the fact that the α-glucoside contains a more strongly *rotating* modification of glucose, the so-called α-*d*-glucose, while in β-glucosides the more feebly *rotating* β-*d*-glucose occurs.

The glucosides are connected with a number of plant pigments. The yellow pigments of many flowers are glucosides in which the carbohydrate groups are combined with the derivatives of the complex aromatic flavone nucleus. The anthocyans, giving a blue or red color to the cell sap of plants, according to the investigations of Willstätter and his collaborators, are combinations of sugars with anthocyanidines—colored substances related to flavones but of somewhat different structure. The anthocyanidines are ampholytes, *i.e.*, they show simultaneously basic and acid properties. Their combinations with bases are blue; with acids, red. This explains the change in their color with change in acidity.

67. Enzymatic Splitting of Polysaccharides. Amylase (Diastase) and Cytase. Hydrolysis of Disaccharides and Glucosides.—The germination of seeds and other food-storage organs is always accompanied by rapid hydrolysis of the polysaccharides present. Let us see what happens to the most important of these reserve substances, starch. Microscopic observations have shown that from the first days of germination, starch becomes subject to an intensive chemical transformation. On the surface of the grains of starch first appear small depressions, which gradually deepen into the grain. By the uniting of these depressions and the formation of cavities, the starch grain finally becomes so tunneled that it falls apart into minute granules, which in the end are completely dissolved (Fig. 90). In place of starch there now appear sugars, namely maltose and glucose. It is easily possible to detect this accumulation of sugar by special reagents, such as Fehling's solution, or simply by tasting. The ungerminated grain of barley has a mealy taste, while the germinating one is sweet, hence, in a dry state, it is called malt.

The germinating seed does not have at its disposal those agents, such as strong acids and high temperatures, by which the hydrolysis of polysaccharides is brought about in a laboratory. It does, however, have substances capable of bringing about this hydrolytic splitting even at ordinary temperature. These substances are known as enzymes. One of the first substances of this sort to be discovered in plants was the enzyme which brings about the decomposition of starch, known as "amylase," or "diastase."

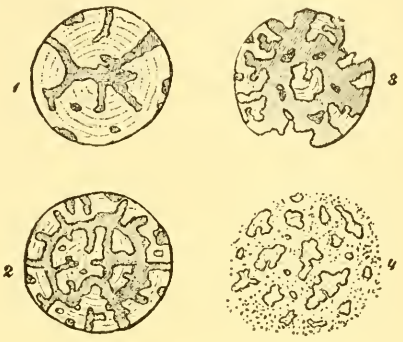

FIG. 90.—Successive stages in the digestion of a starch grain by diastase (*after Beneckc-Jost*).

Diastase can be easily obtained from germinating grain, or malt. The finely ground dry malt is soaked in water, the resulting decoction is filtered, and the filtrate then precipitated with alcohol. The white, flaky precipitate is then filtered off and again dissolved in water. When added to a starch paste, this solution will hydrolyze the paste rapidly. After a short time, the blue iodine coloring characteristic of starch, begins to change: first to violet, then to red, then to a yellowish color, and finally the solution ceases to give the iodine reaction, but, with Fehling's solution, will show a distinct test for sugar. A detailed chemical analysis will show that instead of starch, maltose is present.

The action of diastase, accordingly, differs somewhat from the behavior of inorganic catalyzers, such as strong acids, which attack the molecule, or, more exactly, the colloidal aggregate of starch and rapidly break it up into the end product of hydrolysis, glucose. Whereas diastase acts more slowly, as is indicated by the gradual disappearance of the color reaction with iodine,

the whole process usually stops at the maltose stage. Diastase reduces step by step the colloidal aggregate which we call starch, in other words, it increases the degree of its dispersion. It produces first the intermediate products of the breaking-up process, the dextrines (having properties very close to, if not identical with, glycogen), and then maltose, where its action ceases. It is not able to split maltose into glucose.

Besides its gradual action, diastase differs from inorganic catalyzers also by its specificity. It can act only upon starch, but produces no effect on the reserve celluloses, which are hydrolyzed by acids with no greater difficulty than is starch. Likewise it has no effect either on inulin or cane sugar.

Only a very small quantity of diastase is present in dormant seeds; hence, during the early stages of germination, the decomposition of starch proceeds very slowly. At the time of swelling, however, the quantity of diastase in the seed increases noticeably, and during the first few days of germination it shows a marked increase. At the end of the first week of germination the amount of diastase is usually three to four times as great as it was at the completion of swelling. This increase of diastase with the progress of germination takes place only in the presence of a sufficient supply of oxygen and is most closely connected with the very active respiration shown by germinating seeds. If the seeds are deprived of oxygen, the accumulation of diastase ceases at once.

In the grain of cereals, such as wheat and corn, the centers of formation of diastase are predominantly the embryo of the seed, especially its scutellum (Fig. 91), and the aleurone layer surrounding the endosperm. Whence it diffuses into the tissue of the endosperm, causing decomposition of the starch stored in it. The endospermal tissue proper appears to be lifeless. It is interesting to note that the conversion of starch into sugar in the endosperm proceeds to completion only if the starch remains in close contact with the scutellum and the young sprout adjacent to it, which continually absorbs and utilizes the sugar formed during the hydrolysis of starch. If these parts are withdrawn, the process of the transformation of starch in the endosperm stops quickly as a result of the accumulation of the soluble end products of the reaction.

Simultaneously with the decomposition of starch, the cell walls of the endosperm also are dissolved at germination. Formerly it

was supposed that this was accomplished by diastase. More detailed research has proven, however, that another enzyme, cytase, is at work here, which can be obtained in a comparatively pure state from the germinating seeds of lupine or the stones of the date palm. Like diastase, cytase is formed largely by the embryo. The dissolving of the thickened walls of the endosperm of the date palm stops when the embryo is removed.

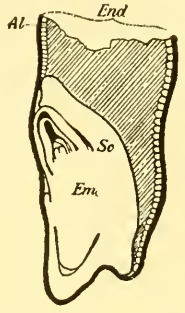

FIG. 91. — Longitudinal section through the lower portion of a grain of wheat (diagrammatical). *End*—endosperm, *Al*—aleurone layer, *Em*—embryo, *Sc*—scutellum (*after Beneckе-Jost*).

Besides these polysaccharide splitting enzymes, there are others at work during germination which hydrolyze the disaccharides and glucosides. Thus, in the conversion of starch into sugar, along with diastase, often maltase is present, which converts maltose into 2 molecules of glucose. Another very widely distributed and much studied enzyme is invertase, which splits cane sugar into glucose and fructose by a process called inversion. It is especially easy to obtain invertase from yeast, the presence of which leads to hydrolysis of the otherwise not fermentable sucrose. When yeast is triturated with pure quartz sand and a small quantity of warm water and filtered, a perfectly transparent filtrate showing a strong invertase action will be obtained. After precipitation by alcohol, a dry preparation of invertase readily soluble in water and preserving its action for a rather long time, is secured.

Of the enzymes which split glucosides, the most important is emulsin or β-glucosidase, which hydrolyzes all the glucosides of the β-group. It is usually obtained from seeds of bitter almonds. The enzyme α-glucosidase, closely related to it, acts in the same manner on α-glucosides. It is found along with invertase in the extract from yeast.

68. General Properties of Enzymes. Methods of Isolation and Purification.—Diastase and cytase belong to the large and very important group of organic catalyzers known as enzymes. Catalyzers are substances which, without themselves taking part in a chemical reaction, speed up the reaction by their presence. They are not found among the end products of a reaction, and they are not used up in the course of the reaction, although their par-

ticipation in the intermediate steps of a reaction is highly probable. A typical inorganic catalyzer is spongy platinum, which, in the production of sulphuric acid by the contact method, can oxidize an unlimited quantity of sulphur dioxide without itself undergoing any special change. In the main, its behavior is based on the fact that, due to adsorption, it condenses the reacting gases on its enormous surface and thus considerably accelerates the reaction.

Enzymes are considered to be organic catalyzers of a colloidal nature. Because of their dispersed condition and adsorbing surfaces, they considerably speed up a reaction going on in their presence. Being colloidal substances, enzymes are unstable in many ways. They coagulate when boiled, thus losing their activity. Solutions of enzymes, if left to stand for a long time, likewise lose their activity, in all probability owing to the gradual breaking down of the enzyme. They are easily precipitated in water solutions by various dehydrating substances, such as alcohol or acetone, but may be redissolved. Many substances, like salts of heavy metals, formaldehyde, and other toxic substances, will destroy enzymes, by causing an irreversible precipitation and a loss of their function.

The chemical nature of enzymes still remains unsolved. The usual methods of obtaining them from solutions, precipitation by alcohol or acetone, are the same as methods of precipitating protein substances, together with which they usually precipitate. Because of this behavior, enzymes were for quite a while classified with the protein group. This, however, has been completely discarded as a result of newer researches. Apparently, the general behavior in precipitation is based upon the fact that in their coagulation protein substances carry along with them the enzymes, just as they are known to carry along dyes of a colloidal nature, fine sediment, etc. Upon this ability to remove from a solution various admixtures is based, for example, the well-known use of the white of an egg for the clearing of a turbid liquid.

As a result of the investigations by Willstätter and his colleagues, it has been possible during the past few years to understand somewhat more clearly the chemical nature of enzymes. Willstätter has based his work largely on the method of selective adsorption of enzymes by certain finely ground substances, such as clay and kaolin. Owing to their extremely high adsorptive capacity, enzymes are removed from solutions more readily by these

substances than proteins, resins, or other colloids. By repeated dissolving and absorption, it is possible in this manner to concentrate certain enzymes and to purify them from various admixtures. The best results were secured with invertase from yeast, which may be extracted in a quite pure condition by water. Willstätter was able to increase the concentration of this enzyme 1,600 times. He has shown that such an almost pure preparation of invertase does not contain phosphorus and does not give a reaction for proteins or carbohydrates. But after all, a preparation of this type is not absolutely pure, and an analysis of it does not give a true idea of the chemical nature of enzymes.

The chemical composition of enzymes is not known, therefore a strict definition of them cannot be given. The presence of enzymes is known only by their action. One may judge of their quantity only by the amount of substance which they transform per unit of time. Even in using crude unpurified preparations of enzymes, however, one of their remarkable properties can be noticed; namely, that an insignificant amount of enzyme is able to induce the transformation of an enormous amount of organic substance. This property is still more marked when purified preparations of enzymes are used. Thus, for instance, 1 gm. of invertin may induce the hydrolysis of 1,000,000 gm. of sucrose. It does not follow, however, that the quantity of enzyme plays no essential rôle. On the contrary, if the rate at which hydrolysis proceeds is determined, it will be found that it is proportional to the amount of enzyme used. If a rapid enzymatic disintegration of a substance is required, therefore, it is necessary to apply a large amount of enzyme.

Of the external conditions which exert a great influence on the rate of enzymatic reactions, the temperature and the actual acidity of the medium must be mentioned. The influence of the temperature on the activity of the enzyme has much in common with the influence of the temperature on vital processes. With an increase of temperature the reaction first accelerates, then a certain optimum temperature is reached at which the reaction proceeds at its highest rate. With a further increase of temperature the reaction begins to decrease and, finally, the destruction of the enzyme occurs. In short, the three cardinal points on the temperature curve of enzymatic action are found: the minimum point at which the reaction only begins to show a perceptible activity,

the optimum point at which it proceeds at its highest rate and, finally, the maximum point at which the reaction ceases. (For a further discussion of cardinal points see Art. 89.) An essential distinction of the enzymes from the living protoplasm is the fact that their optimum points lie considerably higher, *i.e.*, at 45 to 50° C. and in some enzymes even at 60° C. The optimum for living cells usually does not exceed 25 to 35° C., while a temperature of 45 to 50° C. is lethal. The complete destruction of the enzymes is attained at a still higher temperature. The usual method of checking the enzymatic action is by boiling.

The actual acidity, *p*H, of the medium exerts a great influence on the work of the enzymes. Each enzyme has an optimum concentration of hydrogen ions, as well as an upper and a lower limit, exceeding which, the enzyme is either wholly destroyed or rendered inactive. This fact was noticed long ago in animal physiology. The proteases of the digestive canal have been divided in two sharply differing groups: the pepsins working in an acid, and the trypsins working in an alkaline medium. In availing oneself of the modern methods of determining the concentration of hydrogen ions it has been possible to establish the optimum *p*H for all of the more important enzymes of the animal and plant. It has been found that the optimum *p*H for pepsin of the gastric juice is 1.2 to 1.6, for the invertase of yeast 4.2 to 4.6, for the diastase of malt 4.3 to 4.5, for emulsin 4.4, for the protease of yeast 6.7 to 8.5, for the trypsin of the pancreas 7.5 to 9.5. The first four enzymes prefer an acid reaction, the last two, an alkaline medium. These values are not absolutely constant. They depend on the temperature, the substance on which the enzyme acts, the presence of certain chemical substances, etc. The position of the optimum may be slightly changed. Still the influence of *p*H on the activity of the enzymes is so great that it is quite impossible to study the action without precisely determining the acidity of the medium. There is no doubt that the acidity is of equally great importance for the enzymes acting in the living cell, and that changes in acidity may induce a sharp acceleration or a retardation in a series of enzymatic processes. It must not be forgotten that the different parts of the same cell may show different acidities. The protoplasm is always somewhat alkaline, while the cell sap is generally acid.

A very characteristic peculiarity of enzymes is the specificity of

their action. It has been shown that starch is converted into sugar only by diastase, and cellulose only by cytase, and that the hydrolysis of two disaccharides, maltose and sucrose, is brought about, respectively, by maltase and invertase. The classification and nomenclature of enzymes are based on this selective action. Enzymes which act upon carbohydrates, fats, and proteins, are usually divided into three large groups; carbohydrases, which hydrolyze carbohydrates; lipases, which split fats and complex esters in general; and proteases, which attack the proteins. The name of an enzyme is usually composed of the name of the substance acted upon plus the suffix *ase*. Only such enzymes that have been known for a long time: diastase, invertase, pepsin, etc., retain their historical names.

The specificity of enzymatic action should not be understood in the sense that each disaccharide or polysaccharide, each protein, each fat, etc., can be hydrolyzed only by a special enzyme. Quite the contrary, it is established beyond doubt that there are fewer enzymes than are often described, and that various proteins, for example, are acted upon by one and the same enzyme. Their specific action consists largely in the fact that each enzyme possesses the ability to break down any bond of a certain character in the complex molecule of organic compounds, and that in the presence of two optical isomers, each enzyme possesses the ability to act only upon one of them.

The question of reversibility of enzymatic action is of great importance and interest. According to some general laws of physical chemistry, catalyzers, accelerating the course of a reaction, do not, as a rule, change the points of equilibrium between the initial and the end products. But the reactions of hydrolysis are, generally speaking, reversible, that is in the case of a large number of products, accompanying the decomposition and release of free water there is also observed the reverse process of condensation. Theoretically, at least, it should be expected that the same enzymes will accelerate both the decomposition and the synthesis of a complex reserve substance in the organism. In the majority of enzymatic reactions, however, as for instance in the decomposition of starch by diastase, this theoretical requirement is not satisfied. And in those cases in which by the aid of an enzyme it has been possible to obtain synthesis, the product, at best, was found to be only an isomer of the initial substance. From a con-

centrated solution of glucose with the aid of maltase, Croft Hill (1898), for instance, obtained not maltose, but isomaltose. Recently, however, the French scientist Bourquelot was successful in synthesizing with the aid of enzymes, a large number of glucosides from the products of their decomposition, and in such a way that the possibility of enzymatic synthesis was irrefutably demonstrated. It can be assumed with a great degree of probability, therefore, that syntheses of polysaccharides, fats and proteins, which are so common in plant cells, proceed under the influence of the same enzymes which cause their decomposition. The conditions under which such enzymatic syntheses take place are not as yet fully known. It seems to be certain, however, that the continuous removal of water from the sphere of the reaction is necessary. Syntheses of this kind most likely take place in maturing seeds, where the reserve substances are found. The hydrolytic products are produced during the soaking and germinating of seeds. Judging from a number of properties, especially from their easy destruction by heat and toxins, enzymes must be closely related to living protoplasm. Still, considering the ease with which they may be dissolved and precipitated, one is forced to classify them with non-living substances. With the aid of more refined methods of killing, such as desiccation, freezing, or the action of antiseptics, such as chloroform, it is possible to kill the cells of plants without destroying the enzymes found within them. Plants, treated by one or another of these methods, according to Palladin are killed. They should be distinguished from plants which have died as a result, for example, of boiling, in which not only the plasma is killed, but all its enzymes as well.

In carefully killed plants, all the enzymes present in their cells continue the work for a time, and therefore this method is very often used in the study of enzyme activity. However, very soon there are revealed in such cells many irregularities and digressions from ordinary enzymatic action, and many of them will cease to work altogether. This probably happens, because in a killed cell coordination between the activities of the various enzymes is lost. Often some of them begin to destroy others, the reaction of the medium exercising a very great influence upon the whole performance. This phenomenon is called "autolysis" or "autodigestion of the cells," in which the proteolytic enzymes usually continue to work longer than the others.

It is not known in just what way the coordination of the work of the enzymes is brought about in the living cell. There are supposed to be present certain so-called antiferments, which may stop the activity of certain enzymes; and coferments, which hasten it. In the living cell substances may also be found of the nature of proferments, which are not active themselves, but which can easily be changed into enzymes. Enzymes therefore are, as Palladin figuratively expressed it, the "day laborers" of the protoplasm, produced by it and set to work according to its need, and then inhibited or destroyed when there is no need for their action.

Besides hydrolysis, enzymes also bring about other more complex reactions, such as oxidation and reduction of organic substances. These reactions will be discussed in the next chapter.

69. Fats and Their Utilization in Germination. Lipoids and Phosphatides. Organic Acids.—Fats and oils are found in almost all seeds. They enter into the composition of the protoplasm of embryonic cells. But in most seeds they are found in considerable quantity as reserves readily available in germination.

Fats, as is known, are complex esters of glycerine and fatty acids either of the saturated or of the unsaturated series having the general formulae $C_nH_{2n}O_2$, $C_nH_{2n-2}O_2$, $C_nH_{2n-4}O_2$. Saturated acids, such as stearic and palmitic, give solid fats, while unsaturated, as oleic, linoleic and linolenic, are mostly liquid fats. Natural oils, found in seeds, are composed mostly of a mixture of different fats. In this connection it is of interest to note that in plants of a warm climate fats with a higher melting point predominate, as, for example, in the oil of cacao, cocoanut, and other tropical products; while in plants of a temperate zone, such as hemp, flax, poppy, and others, liquid or unsaturated fats predominate. Unsaturated fats are easily oxidized in the air, usually forming a hard film on the surface, hence, they are called drying oils and are used in the manufacture of oil paints. Through the action of alkalies and acids, fats are easily hydrolyzed even at low temperatures, adding 3 molecules of water and breaking up into glycerine and a fatty acid. The hydrolysis of palmitin, for instance, proceeds according to the following formula:

$$\underset{\text{Palmitin}}{C_3H_5(C_{16}H_{31}O_2)_3} + 3H_2O \rightarrow \underset{\text{Glycerine}}{C_3H_5(OH)_3} + 3\underset{\text{Palmitic acid}}{C_{16}H_{32}O_2}.$$

This process is known as "saponification." It is applied in industry in the manufacture of soap and glycerine.

As reserve substances, fats have a number of important advantages over carbohydrates. Being insoluble in water, they readily become inert reserve substances, thus entirely disappearing from the sphere of other reactions and processes going on in the cell. Being liquids, they can completely fill the tissues of reserve organs. Moreover, they do not contain hygroscopic water, of which 15 to 20 per cent is present in reserve carbohydrates as a useless ballast in dry seeds. Finally, they contain very little oxygen, and consequently upon oxidation develop a large amount of heat energy per unit weight. When 1 g. of fat is oxidized in the organism, or outside of it, there is obtained on the average 9.3 cal. of heat while 1 g. of protein will give 5.7 cal. and 1 g. of starch only 4.1 cal. These properties make oil an extremely valuable reserve substance, and hence it is but natural that in 90 per cent of all plants the seeds belong to the oily type. The only advantage possessed by reserve carbohydrates lies in the fact that without any complex chemical changes they can be converted into glucose and the monosaccharides in general, substances of ready availability and great usefulness to plants, while fats must undergo many transformations before they may be utilized.

In the germination of oily seeds, the stored fat is used very rapidly. Müntz observed that of 8.9 g. of fat found in 20 g. of poppy seeds prior to their germination, after 2 days there was left 6.8 g. and after 4 days only 3.9 g. This consumption of oil is preceded by its saponification, which may be seen from the fact that the quantity of free fatty acids, which is quite negligible in the seeds, grows very rapidly at germination. In the case of the germinating poppy seeds only 10 per cent of the fat was composed of free fatty acids before germination, but after 2 days this had increased to 53 per cent, and after 4 days to 97 per cent, in other words, almost half of the fat was already hydrolyzed. It was not possible to demonstrate an accumulation of glycerine, as it very quickly undergoes further changes.

The saponification of fats in germinating seeds is brought about by special enzymes, known under the general name of lipases. Lipase is easily obtained by grinding with glycerine germinating castor beans. It will rapidly hydrolyze fats into glycerine and fatty acids. Since the rate of the reaction is con-

siderably increased in an acid medium (*p*H5), and since in saponification free acids accumulate, the activity of lipase has an autocatalytic character. In the beginning it proceeds rather slowly, and then with ever-increasing rapidity.

The transformation of fats in germinating seeds is not limited to their hydrolysis and the accumulation of fatty acids. The latter very quickly undergo further changes, and in their place sugars appear in considerable quantity. Thus, in one of Frankfurt's experiments the seeds of sunflower contained 55.3 per cent of fats, 3.8 per cent of sugars, while in the sprouts, there had remained 21.8 per cent of fat, but the sugars had increased to 13.1 per cent. In the more advanced stages of germination the surplus sugar may even be transformed into starch.

The process of the transformation of fatty acids into sugars in the germination of seeds generally proceeds very easily in plants just as does the reverse process in maturing seeds—the formation of fat from carbohydrates. It has not been possible as yet, however, to produce this transformation artificially, its chemical nature remaining unsolved. Basically, this transformation of fats into sugars is an oxidation process and is accompanied by the absorption of oxygen, while in ripening seeds a reduction process takes place. The intermediate products of these processes apparently are aldehydes and oxyacids.

A group of substances closely related to the fats are the lipoids, or fatlike substances. The most important representative of this group is lecithin, a complex ester composed of glycerine, two fatty acids, and phosphoric acid, which in its turn is united with a strong, nitrogen-containing, organic base. The general formula of lecithin is:

$$CH_2(OOCR)\cdot CH(OOCR)\cdot CH_2\cdot O\cdot PO(OH)\cdot O\cdot C_2H_4\cdot N\begin{matrix}(CH_3)_3,\\ OH\end{matrix}$$

in which RCOO represent the remains of various fatty acids. In hydrolysis, lecithin splits into fatty acids, choline,

$$\begin{matrix}(CH_3)_3\\ \\ OH\end{matrix}\!\!>\!N\cdot C_2H_4OH,$$

and glycero-phosphoric acid, $CH_2OH\cdot CHOH\cdot CH_2\cdot O\cdot PO(OH)_2$. The various lecithins differ from each other in respect to the

attached acid radical. Under the influence of lipase, lecithin, likewise, gives rise to fatty acids. Phosphoric acid is split off from glycerine by a special enzyme, glycero-phosphatase.

Lecithins are found in all living cells, animal as well as plant. They are not reserve substances, but enter into the composition of the protoplasm as a necessary integral part of it. There is reason to believe that they accumulate especially on its surface, in the so-called plasma membrane. Overton, as has already been noted, attributes an outstanding function to them in the determination of osmotic properties of this membrane. Some authors, for instance, Palladin, likewise attribute to lecithins and to other closely related substances, the so-called phosphatides, an important rôle in the oxidation processes during respiration. Finally, having the capacity to form easily complex compounds with a great variety of substances, particularly with proteins, lipoids may have an important rôle in the synthetic processes of the cell. All these, however, are only suppositions of a hypothetical nature. There are no exact experimental data extant which would indicate the significance of lipoids and phosphatides.

Dibasic organic acids are extremely widespread in the cell sap of plants. They are in the free condition, as well as in the form of salts. The most common of them are: oxalic acid, COOH—COOH; malic, COOH—CH_2—CHOH—COOH; and tartaric, COOH—CHOH—CHOH—COOH. Of tribasic acids the most widespread is citric acid, COOH—COH (CH_2—COOH)$_2$. Many organic acids are found in the fleshy leaves of succulents, as well as in such plants as the sorrel, *Oxalis*, etc. Immature fruits are also rich in these acids. For some time, the view was held that acids originate through the imperfect oxidation of sugars during the respiration process, but this view has been disputed. It appears probable that the organic acids arise from the products of disintegration of proteins, the amino acids, through the splitting off of the amino groups (compare Art. 71). The presence of organic acids is responsible for the acid reaction of the cell sap, which is shown by the majority of plants. In some plants the *p*H value may reach 1.6 to 1.3. In the presence of calcium ions, which are always found in plants, oxalic acid produces crystals of calcium oxalate of different forms: single, clusters, and raphides. The appearance of these crystals is usually a sign of senescence of the cell, they being especially abundant in dying tissues. The almost

instantaneous formation of a great number of small calcium oxalate crystals in the protoplasm, when it is under the influence of ultra-violet rays, has been observed by Nadson. In all probability, this is connected with the rapid disintegration of the compound protein substances of the protoplasm.

70. Reserve Proteins and Products of Their Hydrolytic Decomposition.—The nitrogenous reserve substances of plants are represented almost exclusively by proteins. This group of compounds is of an extremely complex composition, consisting of at least five elements: carbon, oxygen, hydrogen, nitrogen, and sulphur, and often phosphorus as well. Nitrogen constitutes 15 to 19 per cent of the protein molecule. In many respects, protein substances are analogous to polysaccharides. They are represented by large molecules, of a definitely colloidal character, and they are comparatively easily hydrolyzed into more simple, easily crystallizable products, which belong to the group of amino acids; hence, the protein molecule must be considered as consisting of a number of amino acids joined to each other through the process of dehydration, just as we think of the starch or cellulose molecule as being composed of molecules of glucose united to each other. The essential difference, however, is that in splitting polysaccharides only one or two monosaccharides are formed, while at least ten or more amino acids enter into the building of the protein molecule. Owing to this complex composition, the protein group is much larger than that of polysaccharides.

For a long time protein substances were regarded as characteristic integral parts of living matter. It was supposed that in them was to be found the riddle of life. Therefore, the possibility of synthesizing proteins was considered almost akin to the possibility of artificially creating a living organism. At the present time, proteins are not viewed in this light. To be sure, these substances are still considered as absolutely necessary for the creation of the living protoplasm; however, they are no more necessary than are the lipoids or carbohydrates. The protein nature of enzymes, for some time considered quite probable, is now refuted by the majority of investigators. For a long period, there prevailed the theory of the extreme lability of the protein molecule, *i.e.*, that in the course of the life processes it must necessarily be broken down and built anew. But at the present time, this theory also has been abandoned.

Reserve proteins usually are stored in seeds in the form of special bodies, called "aleurone grains." Essentially, they are dried-up vacuoles, very rich in soluble proteins. In many aleurone grains, it is possible to see distinctly well-defined crystals of protein substances, from which it is apparent that colloidal substances may have the capacity to crystallize. In underground food-storage organs, as in the tubers of the potato, reserve proteins are often found in a crystalline form.

Protein substances can be easily detected by the aid of color reactions, of which the most important is the biuret test. In this particular reaction, proteins give a blue-violet or red-violet color upon the addition of a solution of sodium hydroxide and a few drops of a weak solution of copper sulphate. Upon heating with strong nitric acid, proteins give a bright yellow color (xanthoproteic reaction), and upon boiling with mercuric nitrate in the presence of nitrous acid, a brick-red color (Millon's reaction).

It must be noted, however, that all these reactions, except the biuret, are not tests for the whole protein molecule, but only for some of its more characteristic integral parts. These parts, however, may be studied best by way of the hydrolytic decomposition of the protein molecule. For this purpose, the protein is subjected to a prolonged boiling with mineral acids, thus breaking it down into the constituent amino acids. Almost always these are α-amino acids, in which NH_2 is linked to the same carbon atom to which is attached the COOH group. Otherwise, their carbon atom configuration is quite varied, being not only of the aliphatic, but also of the aromatic series. All of them, with the exception of glycine (α-amino-acetic acid), are optically active.

The main amino acids found in proteins are the following:

A. Aliphatic series:

1. Monoamino acids:

a-Glycine, $CH_2HN_2 \cdot COOH$

d-Alanine, $CH_3 \cdot CHNH_2 \cdot COOH$

l-Leucine, $\begin{matrix} CH_3 \\ \\ CH_3 \end{matrix} \!\!\rangle CH \cdot CH_2 \cdot CHNH_2 \cdot COOH$

l-Aspartic acid, $COOH \cdot CH_2 \cdot CHNH_2 \cdot COOH$

d-Glutamic acid, $COOH \cdot CH_2 \cdot CH_2 \cdot CHNH_2 \cdot COOH$

2. Diamino acids:

d-Lysine, $CH_2NH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CHNH_2 \cdot COOH$

d-Arginine, $\begin{matrix} NH_2 \\ \diagdown \\ \\ \diagup \\ NH \end{matrix} \!\!> CNH \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CHNH_2 \cdot COOH$

3. Oxyamino acids:

l-Serine, $CH_2OH \cdot CHNH_2 \cdot COOH$

4. Thioamino acids:

l-Cystine, $COOH \cdot CHNH_2 \cdot CH_2S \cdot SCH_2 \cdot CHNH_2 \cdot COOH$

B. Aromatic series:

1. *l*-Phenyl alanine, $C_6H_5 \cdot CH_2 \cdot CHNH_2 \cdot COOH$
2. *l*-Tyrosine, $C_6H_4OH \cdot CH_2 \cdot CHNH_2 \cdot COOH$

C. Heterocyclic compounds:

1. *l*-Proline, $(C_4H_8N) \cdot COOH$
2. *l*-Tryptophane, $(C_8NH_6) \cdot CH_2 \cdot CHNH_2 \cdot COOH$
3. *l*-Histidine, $(C_3N_2H_3) \cdot CH_2 \cdot CHNH_2 \cdot COOH$

A detailed study of proteins and their products of hydrolysis would be out of place here. Further discussion of this subject can be found in text books of organic or physiological chemistry. It should be pointed out, however, that it is the aromatic nucleus of tyrosine which gives the xanthoproteic and Millon's reactions. Cystine is the typical compound in which form sulphur enters into the composition of the protein molecule, and from it is obtained hydrogen sulphide, which is always formed during the complete decomposition of proteins in the process of putrefaction. Among the products of hydrolysis of proteins, besides amino acids, a small amount of ammonia, and often carbohydrates also are found.

The percentage relationship of various amino acids varies in different proteins, and it is exactly these differences in the relationship of amino acids which are the basic cause of the enormous variety of protein substances. Thus, for example, the reserve proteins from the seeds of some agricultural plants contain the following quantities in percentage of the most important amino acids and ammonia:

	Legumins of Peas	Phaseolin of Kidney Beans	Gliadin of Wheat	Edestin of Hemp
Alanine	2.08	1.80	2.00	3.60
Leucine	8.00	9.65	5.61	20.90
Proline	3.22	2.77	7.06	1.70
Phenyl alanine	3.75	3.25	2.35	2.40
Tyrosine	1.55	2.84	1.20	2.13
Aspartic acid	5.30	5.24	0.58	4.50
Glutamic acid	16.97	14.54	37.33	14.07
Arginine	11.71	4.89	3.16	14.17
Lysine	4.98	4.58	0.0	1.65
Histidine	1.69	2.62	0.61	2.19
Ammonia	2.05	2.06	5.11	2.28

All of these amino acids are obtained from proteins through hydrolysis, apparently without any great changes in the structure of the molecule, which leads to the belief that they are linked to each other in such a way that the COOH group of one acid unites with the NH_2 group of another. It must be remembered that amino acids are amphoteric compounds, able to react as weak acids and as weak bases at the same time. As a result of such unions, there accumulates in the protein molecule a number of CO—NH—CH groups which are the ones that give the biuret test. An amino acid by itself will not give this reaction.

Basing his work upon these suppositions, Emil Fischer (1906), attempted to bring about a synthesis of the protein molecule. Binding together amino acids, first by twos, then by threes, and finally as many as 18 or 19, he obtained complex compounds named by him polypeptides, which gave the biuret reaction and, in general, possessed many of the properties of proteins. The structure of the polypeptide may be represented by the following chain formula:

$$\begin{array}{l}—NH—CH—CO—NH—CH—CO—NH—CH—CO—NH—CH—CO,\\ \qquad\quad|\qquad\qquad\quad|\qquad\qquad\quad|\qquad\qquad\quad|\\ \qquad\quad R_1\qquad\qquad R_2\qquad\qquad R_3\qquad\qquad R_4\end{array}$$

where R_1, R_2, etc. are parts of different amino acids, connected with the link CH of the main chain to form lateral chains. According to the theory of Emil Fischer, actual proteins represent nothing

else than very complex polypeptides constructed in the same manner as his artificial polypeptides.

The investigations of Emil Fischer have made an epoch in the study of proteins, and the above scheme for the structure of a protein molecule was for a long time generally accepted.

Recent researches, however, have shown that besides such peptide bonds in the protein molecule, as well as in the molecule of polysaccharides, there also exist bonds of an anhydrous character, leading to the formation of ring, not open chain, compounds. This forces one to conclude that the protein molecule is of a more complex structure than it seemed immediately after the brilliant work of Emil Fischer. This does not mean, however, that the protein molecule is extremely large. On the contrary, there is reason to believe that in colloidal solutions proteins, as well as polysaccharides, form aggregates of many molecules, and that the size of each molecule consists not of thousands, as was thought until recently, but only of several, or at most of a few hundreds of atoms.

A rational classification of protein substances should be based upon their composition, that is, upon the quantitative relationship of the amino acids entering into their structure. This classification has not yet been worked out, and customarily the old classification, based primarily upon differential solubility of proteins in water and salt solutions, is used.

The main features of this classification are as follows: First they are separated into simple and complex proteins, or proteids. The latter are combinations of a strictly protein molecule, the structure of which we have just learned, with another complex molecule. If this complex is of a carbohydrate character, we obtain glucoproteids, to which belong many of the mucilages; if it is a complex of nucleic acids, we obtain nucleoproteids.

Simple proteins are divided into the following four groups:

1. Albumins—soluble in water.
2. Globulins—insoluble in water, but soluble in weak solutions of neutral salts.
3. Prolamins—insoluble both in water and in salt solutions, but soluble in 70 per cent alcohol.
4. Glutelins—insoluble in all of the above solvents, but soluble in weak alkalies.

Reserve proteins of seeds belong to the group of simple proteins. Of these, albumins are practically unknown in plants. Of

globulins, legumin and vicilin are found in the seeds of peas and some other leguminous plants; phaseolin, in seeds of the kidney bean; conglutin, in those of lupine; and edestin, in hemp seeds. Of prolamins the most important is gliadin, which enters into the composition of wheat gluten; hordein, in the grain of barley; and zein, in the grain of maize. Of the glutelins we should note glutenin, the other part of the gluten of wheat. Reserve proteins of plants have been studied in great detail by Osborne and his colleagues and by Prianishnikov.

71. Changes in Nitrogenous Substances at Germination. Enzymatic Destruction of Proteins and Synthesis of Asparagine.— The protein reserves of seeds, like the polysaccharides, are subject to hydrolysis at germination. This is brought about by special, so-called proteolytic enzymes, or proteases.

The first of these to be studied were the ones present in the digestive tract of animals. Soon two types of them were established: pepsin, secreted by the walls of the stomach and causing a rather weak hydrolysis of proteins into the so-called peptones; and trypsin, secreted by the pancreas and splitting proteins into amino acids. Peptones are quite complex bodies of a colloidal nature, but the degree of their dispersion is considerably greater than in the true proteins. It is usually thought that peptones are the first products of the decomposition of the protein molecule, but recently evidence has accumulated which shows that in the formation of peptones the protein molecule itself is not decomposed, but only the larger colloidal aggregates, composed of several molecules, are broken down. Peptones are not fully determined chemical compounds.

Proteolytic enzymes, or proteases, also can be found in plants. The most active and best-known plant protease is papain, obtained from the juice of the fruit of the papaw tree (*Carica papaya*). The isolation of proteases from germinating seeds is more difficult than the isolation of diastase. Their behavior is usually studied by employing autolysis, that is "autodigestion" of the protein substances, first by pulverizing the seeds, and then by soaking them in water with the addition of chloroform or some other neutral antiseptic, which prevents the development of microorganisms, and kills the remaining uninjured living cells of the seed embryo. An extremely active protease is found in yeast. During the autolysis of yeast the protein substances are split very rapidly.

This protease, however, in contradistinction to invertase, is not excreted by the yeast into the surrounding medium but acts only within the cells. It has been called, therefore, endotryptase. Since plant proteases belong to the trypsin type, as a result of autolysis, there is secured a mixture of amino acids. In their quantitative composition, they correspond to the mixture which is obtained through the hydrolysis of seeds by mineral acids. There are found in plants also proteolytic enzymes of the nature of pepsin (peptases). They are especially easily detected in insectivorous plants, where they serve in the digestion of captured insects.

The physiological significance of the splitting of proteins during the germination of seeds consists in the fact that these proteins first of all must be transformed into a conveniently movable form, since they must be transported from the reserve tissues, such as the endosperm and the cotyledons, into the growing parts of the developing embryo. Moreover, reserve proteins are only slightly soluble or entirely insoluble, and, even in a dissolved form, they are unable to pass through the cell membranes, owing to their colloidal nature. Another no less important result of the breaking down of proteins into amino acids is their loss of specific characters. Every protein being characterized by a combination of amino acids specific to it, it is not possible therefore to change one protein directly into another. In order to do this, it is necessary first to decompose it into its simplest integral parts and only then may they be combined into a new protein molecule. This is attained by a complete destruction of the protein molecule into the end products of its hydrolysis.

It is especially convenient to observe the disintegration of proteins in seeds when germination takes place in the dark, to prevent the processes of photosynthesis. It has been shown that over two-thirds of the total proteins are of the form of simple compounds. This, in spite of the fact that germination, even in the dark, is connected with the growth of the seedlings, thus producing a considerable increase in the number of living cells and, consequently, an increase in the total mass of living protoplasm. In an experiment with vetch seeds germinating in the dark, Prianishnikov observed that after 10 days of germination 54 per cent of the protein substances found in the grain remained undisintegrated; after 20 days, 40 per cent; after 30 days, 33 per cent; and after 40 days, when the young plants were already beginning to die from exhaustion, 32

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per cent. These figures show that even in case of starvation, a part of the protein substances is preserved. The reserve proteins of the seed were, of course, entirely disintegrated by this time. The less labile protein substances of the protoplasm belong to the group of nucleoproteids. They are complex proteins, representing a combination of a simple protein with nuclein (see Art. 72).

In germinating seeds the breaking down of proteins goes even beyond the stage of amino acids. This complicates considerably the general picture of the transformation of nitrogenous substances at germination. Boussingault (1864) has pointed out the fact that in germinating seeds, especially in legumes rich in reserve proteins, there accumulates a large quantity of asparagine, an amide of aspartic, or amido-succinic acid,

$$CONH_2 \cdot CH_2 \cdot CHNH_2 \cdot COOH.$$

He has expressed the supposition that asparagine is analogous to the amide which accumulates in the blood of animals, namely, urea, $NH_2 \cdot CO \cdot NH_2$, and this forms a way of fixing ammonia, the accumulation of which would expose the organism to the danger of being poisoned with this toxic product.

Boussingault's idea has been substantiated experimentally through the work of Schulze (1875), who showed that, in reality, asparagine is not the initial product of the decomposing protein molecule, since there accumulates much more of it in seeds than there was of aspartic acid in the reserve proteins, and that the increase of asparagine, especially during the later stages of germination, is at the expense of the remaining amino acids. Thus, by comparing the analysis of sprouts of peas 1-week old with those 3 weeks old, Schulze found the following quantitative changes of the most important amino acids and asparagine:

	Leucine	Tyrosine	Arginine	Asparagine
First week.....	Much	Little	Trace	None
Third week.....	Much less	Disappeared	Almost disappeared	Very much

The amount of asparagine accumulating in the seedlings may constitute up to 60 to 70 per cent of the total amount of protein found in the seeds before germination. The highest percentage

of aspartic and the related glutamic acids does not exceed 20 to 25 per cent.

Asparagine, accordingly, is not the initial product of hydrolysis, but the result of synthesis. This view received a final substantiation through the experiments of Prianishnikov. On germinating in solutions of ammonium salts, he observed the synthesis of asparagine in seeds not only from the amino acids present during germination, but also from ammonia supplied from without. In this connection it is interesting to note that not all seeds bring about this synthesis with equal ease. It proceeds readily in seeds rich in non-nitrogenous reserves, such as the carbohydrates and fats, for example, in the seeds of graminaceous plants. In seeds of leguminous plants, in which there are large amounts of reserve proteins, but the carbohydrate content is comparatively small, asparagine can be synthesized from ammonium salts only when calcium carbonate is supplied at the same time, which neutralizes the free acid. But the seeds of lupine, which are very poor in non-nitrogenous reserves, are unable to form asparagine from ammonia, since the necessary carbohydrates are lacking.

Prianishnikov's data illustrate the different capacities of seeds for synthesizing asparagine from the absorbed ammonium compounds. In the table are given amounts of asparagine, in milligrams, which formed in the dark in 100 seeds of each type of plants. In the first case, germination was conducted in water; in the second instance, in a solution of ammonium chloride; in the third, in a solution of ammonium chloride with the addition of calcium carbonate.

Conditions of Germination	Barley	Pumpkin	Vetch	Pea	Lupine
Water	37	194	76	258	258
NH_4Cl	56	379	73	283	175
$NH_4Cl + CaCO_3$	.....	.....	118	441	158

In the lupine there was a decrease of asparagine synthesis in the presence of ammonium salts. But when these seeds were given carbohydrates, that is, when they were allowed to germinate in the light, an increase of asparagine syntheses in the presence of

ammonium chloride was observed. This is formed from the ammonium absorbed from the nutrient solution.

Prianishnikov has expressed it figuratively by saying that ammonia must be recognized as the alpha and omega of the transformation of protein substances in plants. It splits off from amino acids, appearing as the final end product of protein decomposition. Similarly, it is the initial substance from which asparagine is produced and from which the synthesis of nitrogenous compounds in plants begins. In some plants rich in proteins but poor in non-nitrogenous substances, especially the fungi, instead of asparagine, urea is the intermediate product of protein exchange, so that in this respect they are still closer to the animal organism than higher plants.

Large quantities of urea are accumulated in the fruiting bodies of Lycoperdon, in which it constitutes 10 per cent or more of the total dry weight. In plants whose cell sap is very rich in organic acids, the ammonium salts of these acids are accumulated instead of asparagine.

This analogy between asparagine and urea emphasizes a very important physiological difference between animals and plants. Animals fix ammonia, resulting from the complete disintegration of proteins, in the form of urea, and excrete it from their bodies as unnecessary refuse. It is of no use to the animal organism, which cannot bring about the reverse synthesis of amino acids and proteins from ammonia and non-nitrogenous substances. Plants on the contrary, having high synthetic capacity, fix ammonia in the form of asparagine, which remains unaltered in the body of the plant and then serves as the foundation material for further syntheses, splitting off ammonia when needed.

72. Proteins of the Protoplasm and the Nucleus. Synthesis of Protein Substances in Plants.—Asparagine is the initial product of the synthesis which always accompanies the hydrolysis of protein substances at germination. Germination is really a double process, consisting of the breaking down of stored substances and of their resynthesis into material to be used for the newly developing parts of the young plant. Thus, in the transformation of carbohydrates at germination the breaking down of starch and other polysaccharides into the simple sugars is followed by the synthesis of cellulose, which serves for the building of membranes of the new cells. The same is true of proteins. They are hydrolyzed, while the

crystalline products of their hydrolysis, the amino acids, appear. These in turn give rise to ammonia. This stage, however, is followed by synthetic processes. Ammonia changes into asparagine, which, together with non-nitrogenous substances, forms new protein compounds, thus making the protoplasm of the new cells.

The protein substances entering into the composition of the nucleus and the cytoplasm differ quite radically from the reserve proteins of the seed. Many of them belong to the complex proteins. The most studied of these are the nucleoproteins, which evidently form the main part of the chromatin of the nucleus. Nucleoproteins are very complex compounds. The pepsin of the gastric juice removes the protein group from these compounds, leaving the indigestible nuclein. By further hydrolysis, the nuclein can be broken down by splitting off another protein group and leaving only nucleic acid, which has nothing in common with proteins. Finally, by still more thorough hydrolysis, the nucleic acid itself can be broken down to phosphoric acid, a carbohydrate group, and pyrimidine and purine bases.

Purine and pyrimidine are heterocyclic compounds. Nucleic acid contains two purine derivatives, adenine and guanine, and two pyramidine derivatives, cytosine and thymine. All of these being substances of a basic character, they therefore will combine readily with phosphoric acid. The carbohydrate group is a remnant of glucose. Plant nucleoproteids contain uracil instead of the thymin of animal proteins, the pentose ribose, $C_5H_{10}O_5$ and *l*-xylose, $C_5H_{10}O_5$. In the breaking down of nucleoproteins in the animal organism the derivatives of purine most often give uric acid.

The proteins of the protoplasm have been studied very little. In protoplasm, glucoproteins are often found—complex proteins which split off the carbohydrate group on hydrolysis. Fungi are especially rich in glucoproteins. Protoplasm contains also special phosphorus-containing proteins, which do not belong to the nucleoproteins and are called plastins. Many authors, including Lepeschkin, suppose that the proteins of protoplasm, though of a very complex structure, are chemically bound still further with lipoids forming molecular complexes of an enormous size and capable of the most varied biochemical reactions. It was at one time supposed that "living" proteins of the plasma differ from "non-living" ones in that nitrogen is found in them not in the form of the amino

group, NH_2, but in the form of the cyanic group, C:N, which gives them a special instability and high capacity of transformation; two essential properties of life. This theory was first advanced by Pflüger, and was supported for a long time by Loew and Bokorny, who by means of a special aldehyde reaction attempted to distinguish chemically between living and non-living proteins. The most recent biochemical investigations do not support this idea, and therefore we have no reason to believe that the proteins of protoplasm are especially unstable. Quite the contrary, in their relation to enzymes and to many chemical reagents they are usually more stable than the reserve proteins.

In general, the chemical composition of protoplasm has not yet been sufficiently studied, since it usually occupies only an inconsiderable part of the whole volume of the cell, and is very difficult to separate from the substances found in the cell sap.

The most convenient objects for the study of protoplasm are the plasmodia of slime molds (Myxomycetes), which are large aggregates of naked protoplasm. A careful analysis performed comparatively recently by Kiesel (1925) gives the following percentage composition of the plasmodium of one of these molds (see table below).

In this instance, of course, not only the active protoplasm, but also a number of reserve substances have been included, as evidenced by the presence of glycogen and certain characteristic fractions of the fats and proteins. A full separation of the reserve substances from the active ones, however, is hardly possible, since for their work the active parts of protoplasm continually require reserve substances, which are always present within the cell.

	Per Cent
Fats	17.85
Lecithin	4.67
Cholesterol	0.58
Mono- and disaccharides	8.06
Polysaccharides (largely glycogen)	17.02
Soluble nitrogenous substances	12.00
Proteins (including those of the nucleoproteins)	20.65
Plastin	8.42
Nucleic acid	3.68
Fats from lecithoproteins (?)	1.20
Unknown substances	5.87
Total	100.00

Not having a complete idea of the composition of the active proteins of protoplasm, we cannot understand clearly the process of their synthesis. At any rate their synthesis begins with the end product of the hydrolyzed reserve proteins; with ammonia. Besides asparagine, which releases ammonia, non-nitrogenous substances, especially the carbohydrates, also take part in this synthesis. With seeds poor in carbohydrates, as lupines, germination experiments in darkness have definitely shown that a large quantity of asparagine accumulates, but that the synthesis of protein substances is at a standstill. When such sprouting seeds are placed in light, a rapid synthesis of proteins is soon initiated through the utilization of carbohydrates which are produced by the leaves in light.

Light really plays an indirect part in this instance. Seeds rich in carbohydrates are able to synthesize proteins in darkness as well, and they do not accumulate any large amounts of asparagine. Similarly, the synthesis of proteins in the germinating bulbs of the onion and other closely related plants will proceed in darkness. It is very convenient to study protein synthesis in these bulbs, since both the nitrogenous and non-nitrogenous reserves are stored in the form of crystalline substances, as sugars and amino acids. In germinating seeds, on the other hand conclusions about the synthesis of proteins in the protoplasm must be founded on the basis of indirect evidence, namely, by the increase in the quantity of the nucleins indigestible in gastric juice or by the increase in amount of purine bases.

Such processes of hydrolysis of reserve proteins and synthesis of active proteins of the protoplasm also take place at the time of resumption of growth by other storage organs. Asparagine, for instance, accumulates in large quantities in the underground sprouts of asparagus, from which it has received its name. Considerable quantities of it are also to be found in the unfolding leaves and flower buds of trees which have been kept in darkness; but in the light, synthetic processes soon become dominant, proceeding at the expense of the newly produced carbohydrates.

73. Nutrition of Saprophytic Microorganisms and Higher Plants.—Saprophytes do not produce the organic substances necessary for their nutrition. They secure these already prepared, settling wherever there are accumulations of such substances, as in soil rich in humus, on the dead parts of animals and plants, or on

food products. Closely related to saprophytes are the parasites, which feed on living organisms by robbing the nutritive substance created by them.

The principal differences between the nutrition of saprophytes and autotrophic plants are really not so great. All the non-green parts of a plant lead essentially a sort of saprophytic life, receiving nutritive substances from the green parts. In fact, the protoplasm of the green cells themselves receives its main nutritive substance, the carbohydrates, in a prepared form from the green plastids.

Animals, likewise, feed on prepared organic compounds. A very important difference is to be noted, however, between plant saprophytes and animals. Though they do not have the ability to synthesize carbohydrates from carbon dioxide and water, saprophytic plants have to a full extent the characteristic capacity of plants to synthesize protein substances from ammonia and carbohydrates, while some of them can even reduce nitrates. Animals, on the other hand, are entirely incapable of effecting such synthesis.

There are very few saprophytes among the higher plants, most of them belonging to the orchids (*Neottia,* bird's-nest orchid). The cause of this apparently lies in the fact that the roots of higher plants have almost entirely lost the ability to absorb organic substances. All attempts to grow higher plants artificially in sterile solutions of organic substances have resulted in very weak growth; and when kept in darkness such cultures have failed completely.

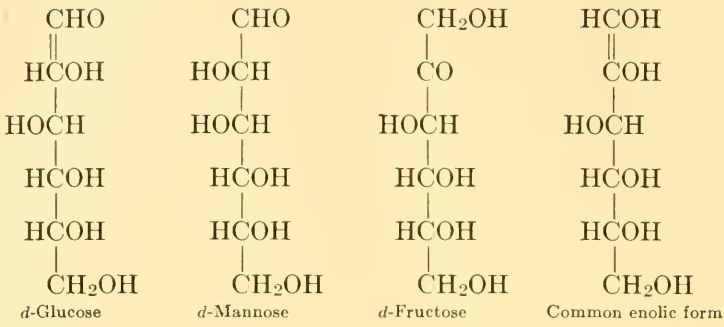
Lower plants, which absorb solutions not through roots, but by their whole surface, are much more adapted to a saprophytic mode of life. Many green algae (*Ulva, Cladophora*), but especially those of the blue-green group, usually supplant their photosynthetic activity by absorbing organic substances from without. They, therefore, may be called facultative saprophytes. The large group of the fungi consists entirely of saprophytes and parasites.

Saprophytic microorganisms may be divided into two physiological groups: Those of the first group are able to use almost all forms of organic substances and can live on practically any disintegrating organic matter. Such are the numerous molds and many bacteria. They play an extremely important rôle in nature. By destroying the dead bodies of plants and animals, and by chang-

ing animal refuse into inorganic form, they make it adaptable to the nutrition of higher green plants, and through them for animals. Organisms of this kind are found in tremendous numbers in the soil, in drainage water, in rivers and lakes, everywhere displaying their life activity. The other group is composed of microorganisms capable of feeding only upon some sharply limited group of organic substances. Some of them—like the vinegar bacteria, thrive only in alcoholic solutions; others feed upon cellulose or upon dextrin or pentoses; still others are capable of assimilating even such inert substances as paraffin, which is entirely unfit for other organisms. Because of their adaptability to certain substances, such saprophytes remind one of the parasites, many of which are likewise adapted only to certain hosts which produce specific substances necessary for their development.

For the nutrition of the omnivorous saprophytes different organic substances are used with unequal readiness, and hence their development proceeds at varying rates. It is impossible to determine the exact needs of nutrition of these plants since even of the omnivorous saprophytes not all have like requirements. Yet a certain order and preference in the consumption of different substances seems to exist. The sugars, especially glucose, are the best sources of carbon, and substances with straight carbon chains are usually preferred to compounds with side chains. The lower members of a homologous series are less utilized by these organisms than the higher ones, and the most readily absorbed compounds seem to be those with five or six carbon atoms. The introduction of hydroxyl groups into the carbon chain likewise raises the nutritive value of a compound. All these interrelationships have their explanation in the fact that before they are used these substances are transformed by the saprophytes into sugars, which are the main nutritive substances for all plants. In general then, the closer the structure of the organic substances is to that of sugars or the easier their transformation into sugars, the more readily are they assimilated.

Highly interesting is the relationship between the nutritive value and the optical structure of some of these compounds. Of the sugars only a certain group is readily transformed; namely, *d*-glucose, *d*-fructose, and *d*-mannose. All these sugars show a related configuration of atomic groups and with intramolecular migration of the hydroxyl they exhibit the same enolic form.



Galactose, showing a somewhat different configuration, is utilized with greater difficulty.

Therefore, substances which are analogous to this group, such as dextrotartaric acid, are assimilated much better than their laevo-rotating optical isomers, which are often left untouched by microorganisms. Of the great variety of organic compounds of different degree of availability plants will always completely utilize the more available compounds and only then begin to use the less assimilable ones. On the different nutritive value of the *l*- and *d*-isomers is founded the biological method of differentiation of racemic compounds, first used by Pasteur (1850) in his classical work on tartaric acid.

Substances of the aromatic series, in general, are utilized with difficulty by saprophytes, but some hydroaromatic ring compounds, as quinic acid, inositol, quercitol, are good nutritive material for molds, since they readily break down and give rise to sugars.

The nitrogen requirements of saprophytes are varied. The interesting and important group of bacteria which are able to assimilate nitrogen of the atmosphere were discussed in Chap. II (Arts. 19 and 20). The majority of saprophytes do not have this capacity, but they can build their protein substances from nitrates, nitrites, and ammonia, of which the latter is a better source of nitrogen for them than either the nitrates or nitrites, since for its conversion into proteins and amino acids considerably less chemical transformation is required. In this connection, it is interesting to note that the nutritive value of various nitrogenous substances to a large degree depends upon the nature of the available carbon compounds. In the presence of sugars, nitrates are utilized more readily than in the presence of mannite or glycerine.

Some organisms, especially bacteria, are not able to use either the NH_2, or NO_3 groups. They require organic nitrogenous substances, and are usually grown by adding peptone to the nutrient medium. The method of nutrition of such organisms, to which belong the lactic-acid bacteria, and also some facultative parasites related to the bacteria of anthrax, is somewhat similar to the nutrition of animals.

The digestion of nutritive substances by saprophytes is similar to the digestion of the initial products of assimilation or the reserve substances by autotrophic green plants. The most important agencies in this work are the enzymes, the energetic action of which in the saprophytes often surpasses that in higher plants. Another peculiarity of the enzymatic action of saprophytes is the secretion by them of enzymes into the surrounding medium. This has an especially important significance in the utilization of insoluble substances, such as starch or proteins, since they require a preliminary hydrolysis before they can be absorbed by the cell of the saprophyte. Such enzymes are called "ectoenzymes," to distinguish them from endoenzymes, which work only inside the cell. Of the enzymes formed by molds, one very well known, and even used in medicine, is Takadiastase. It is secreted by the fungus, *Aspergillus oryzae*, found on rice grains. A preparation of this enzyme will convert starch into sugar with great rapidity, but it likewise will develop a proteolytic activity, liquefying gelatin, which indicates the admixture of a considerable quantity of proteolytic enzymes with this diastase.

An interesting phenomenon is the regulation of the secretion of enzymes in saprophytes. *Aspergillus* or *Penicillium*, commonly used in laboratory work, for instance, are capable of producing the most varied enzymes. They do not always produce them in any definite proportion, but will form predominantly or even exclusively those enzymes which will act upon the substratum on which these organisms are developing at a particular time. Thus, when grown on starch, they will form mainly diastase, but on sugars diastase is not formed at all. On fats they form lipase in large quantities, while on protein substances an energetic secretion of protease takes place. Even when unusual nutrient substances are supplied to them, for example tannin, special enzymes, as tannase, are produced, while normally not a trace of these enzymes seems to be present.

Besides organic nutrients, saprophytic microorganisms likewise require mineral substances. To obtain a good development of bacteria or molds it is necessary to supply approximately the same mixture of anions and cations, as is commonly provided for higher green plants. Particularly necessary are K, Mg, P, S, and Fe, and often (especially for bacteria) Na and Cl. The only exception is Ca, which is neccessary for the nutrition of green plants, but is superfluous for fungi. The necessity of mineral salts for microorganisms was first shown by Pasteur (1860). Through the careful and painstaking work of one of his students, Raulin, the mineral nutrient requirements of the fungus, *Aspergillus niger*, was studied in detail, and the classic composition of the solution necessary for the optimum development of this fungus was given. It is as follows: 70 g. cane sugar; 4 g. tartaric acid (to give an acid reaction); 4 g. NH_4NO_3; 0.6 g., $NH_4H_2PO_4$; 0.4 g., $MgSO_4$; 0.6 g., H_2CO_3; 0.25 g. $(NH_4)_2SO_4$; 0.07 g., $FeSO_4$; 0.07 g., $ZnSO_4$, and 1500 cc. of water. Since then, it has been possible to simplify considerably this solution. Zinc is not among the elements absolutely necessary, but in its presence development proceeds more rapidly and a higher total yield is obtained, but the development of fruiting bodies is somewhat delayed. Raulin's solution shows clearly that molds can develop very well on inorganic compounds of nitrogen, which serve for the synthesis of its organic compounds. This capacity, which is also peculiar to yeasts, was made use of in Germany during the World War, when a decided shortage of protein substances for the diet of the people was felt. Great quantities of yeast, the cells of which are very rich in proteins, were cultivated on sugar solutions with the addition of ammonia or urine, then washed and dried, and mixed with flour. Bread baked from such flour had a higher protein content and a high nutritive value.

74. Nutrition of Parasites. Insectivorous Plants.—As has been noted, there are almost no true saprophytes among the higher plants; but among them are found a number of parasitic and semiparasitic forms, as, for instance, dodder (*Cuscuta*), broom rape (*Orobanche*), mistletoe (*Viscum*, *Phoradendron*), and others, which, although they retain the basic characteristics of an autotrophic plant, particularly the chlorophyll-bearing apparatus, nevertheless secure a part of their food requirements from other organisms.

In the figwort family (*Scrophulariaceae*), especially the genera *Melampyrum*, *Alectorolophus*, *Euphrasia*, *Pedicularis*, and others,

the external appearance is much like ordinary green plants; their roots, however, are very weakly developed and end in haustoria, by means of which they fasten themselves to the roots of other plants. The physiology of these semiparasites has been studied in detail lately by Heinricher, who was successful in growing them in

FIG. 92.—An artificial culture of *Melampyrum arvense* on roots of the hazel (*after Molisch*).

artificial cultures (Fig. 92). It was found that their chlorophyll-bearing organs work normally and that from the host plant they receive only water and nutritive salts dissolved in it, in all probability, mainly nitrogenous substances.

A somewhat higher degree of parasitism is exhibited by mistletoe (*Viscum* and *Phoradendron*, Fig. 93) and other representatives of the family *Loranthaceae*. These plants inhabit the branches of

trees. They send haustoria under the bark, attaching them to the vascular system of the host and absorbing water and mineral salts. Having well-developed and normally functioning leaves, they do not need to obtain carbon compounds from the host. As a rule, mistletoes do not harm trees very greatly, although they do take away from the host part of the mineral and especially the nitrogenous substances (Fig. 94). Hence, when several clusters of mistletoe have settled upon a tree, it may eventually die. It is inter-

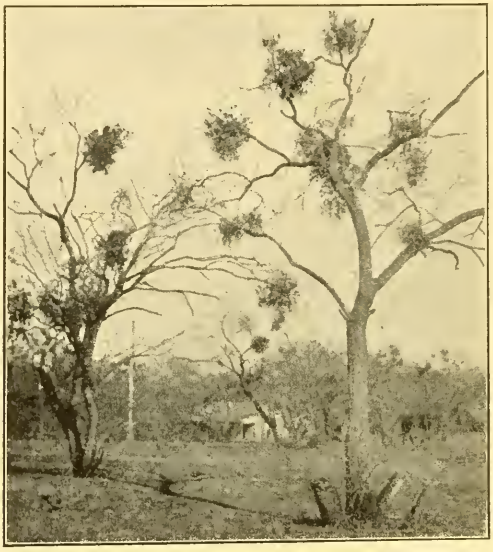


FIG. 93.—Mistletoe on branches of a tree (*after Transeau*).

esting to note that some trees are immune to mistletoe. The seeds of the parasite which happen to fall upon their bark die soon after germination. The cause of this immunity is not known. Others, on the contrary, are unable to withstand the invasion of this parasite.

A very peculiar group of semiparasites are the insectivorous plants. In the quest for the necessary nitrogen compounds they have developed the ability of securing nitrogen from insects and other small animals. They must, of course, first catch and then digest these insects. The organs of capture have a rather varied form and structure. In the sundew (*Drosera*), common in peat bogs, there occurs a secretion of a sticky fluid on special tentacles,

which glues down small insects. This entangling process is followed by a certain bending of the leaves and tentacles, and in the

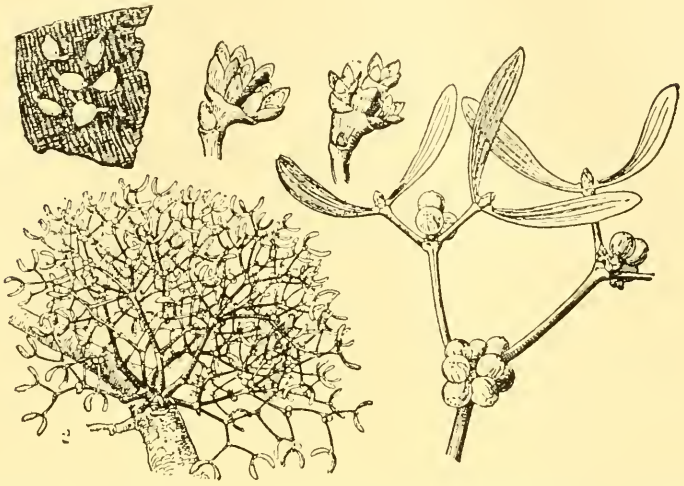


FIG. 94.—To the right, a branch of mistletoe with leaves and fruits. To the left, below, a mistletoe bush which has sent its roots under the bark of the host plant. To the left, above, seeds of mistletoe germinating on bark of host.

end the insect is securely entrapped. In the American plant, Venus's flytrap (*Dionaea*), the leaf is a quickly closing trap (Fig. 95). In *Nepenthes*, insects drown in the liquid filling the lower part

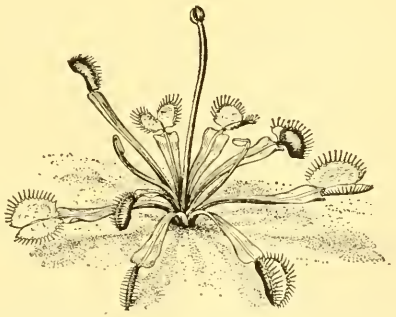


FIG. 95.—Venus flytrap showing traps open and closed (*after Brown*).

of a special urn, which is suspended from the end of the leaf blade (Fig. 96). Whatever the method of entrapping, it is

always followed by digestion of the catch. This is accomplished by the secretion of an acid fluid containing pepsin. The digestion proper resembles stomach digestion. The peptones thus obtained are absorbed by the tissue of the leaf. Once inside the cells, they undergo further hydrolysis and transformation. Only the protein substances of the caught animals are subject to digestion. Fats and polysaccharides are left untouched, thereby indicating that it is the need for nitrogen compounds which has made these plants resort to such an unusual method of nutrition. The natural dwelling places of insectivorous plants, peat bogs for *Drosera* and *Dionaea*, the bark of trees for epiphytic *Nepenthes*, etc., are usually poor in nitrogen, and it was Darwin who noted that sundews, obtaining animal food, developed much better and gave rise to more flowers and fruit than when deprived of insects.

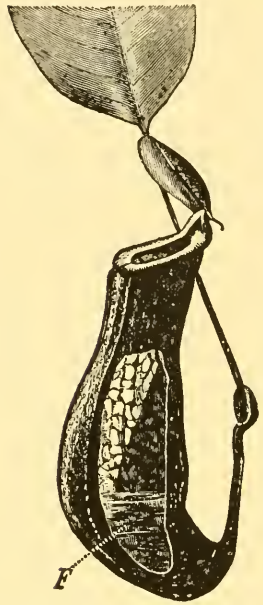


FIG. 96.—Pitcher of *Nepenthes* suspended from the leaf. Note amount of liquid (*F*) within (*after Molisch*).

We find among the higher plants also true parasites, which obtain their nutritive substances wholly from the host plant. Of these, the most common to us are the dodder (*Cuscula*) which at times causes considerable damage to clover fields, and the broom rape (*Orobanche*), which in places makes sunflower culture almost impossible. Dodder, like morning-glory and other plants, entwines the stalk of the host, and in addition, whenever the contact is sufficiently strong, it sends out special suckers (haustoria). Though morphologically representing supplementary roots, by their structure they remind one more of the hyphae of fungi, which penetrate into the tissues of the host, especially into its vascular bundles, and suck from them the nutritive substances (Fig. 97). Dodder does not possess green leaves. Having accumulated sufficient reserves, it promptly sets to flowering and fruiting. Broom rape, quite contrary to dodder, does not attach itself to the aerial parts, but to the roots of the host, and in this respect bears some similarity to semiparasites of the type of yellow rattle. It has no green leaves and produces all its large inflorescences at the expense

of the substances absorbed from the host, and of course, greatly exhausting it in doing so. Both broom rape and dodder infest only certain forms of plants; others are immune. One of the factors upon which depend both the germination and the penetration of the broom rape into the roots of the host is a definite hydrogen-ion concentration, which is close to *p*H 7. In a more alkaline or a more acid medium germination does not take place. The seeds of these parasites, especially of broom rape, are very small and are easily disseminated by wind; besides, they are capable of lying in the ground for many years without germinating; hence, our soils always contain a considerable number of seeds of broom rape, and to combat this parasite is possible only by growing immune varieties of cultivated plants.

Fig. 97.—Dodder entwining the stem of a host plant (*after Brown*).

Still parasitism among higher plants is an exception rather than a rule, but among fungi, which are generally heterotrophic, an enormous number of all kinds of parasites are found, affecting higher as well as lower plants and often attacking animals. Bacteria are primarily animal parasites, although there exists a number of bacterial diseases (bacterioses) of plants, especially in potatoes and tomatoes. The study of plant diseases comprises the special science of phytopathology. We do not have the opportunity to cover this extensive subject here. It should be pointed out, however, that similar to saprophytes, with which they are united by a series of imperceptible transitions, parasites secrete into the surrounding medium, the tissue of the host plant, various

enzymes, which make it easier for the parasite to penetrate into the cell and use the food found therein. The most important of these enzymes is cytase, which dissolves the cell membrane in the path of the parasite. Closely related to the enzymes are the toxins, special substances which poison the host, thus lowering its resistance to the attack of the parasite.

In general, parasites are rather narrowly adapted to particular nutritive substances, which they find only in certain plants. The cause for this specialization is not yet understood. It is possible to change many of them to a saprophytic life by cultivating them on broth or infusions from tissues of the host plant.

The relationship between many trees and shrubs and certain fungi thickly enveloping their roots and even penetrating into their cells, is an odd phenomenon and very little understood at present. These fungal formations are called mycorrhizae. When the hyphae of the fungus cover the root and penetrate only into the intercellular spaces of its bark, we have an ectotrophic mycorrhiza. These are distinguished from the endotrophic form, in which the hyphae of the fungus penetrate into the interior of the cells of the root and become firmly established therein. The endotrophic mycorrhizae are widespread among the orchid family, where they form such a permanent integral part of the host plant that some authors are inclined to compare orchids to lichens and view them as complex organisms, composed of a fungus and a higher plant, just as the lichens are composed of a fungus and an alga.

In some cases the symbiotic relation is even hereditary, as the fungus hyphae penetrate into the seeds and at germination begin to grow with the growth of the first rootlet. Notwithstanding such a close tie between fungi and roots, the significance of mycorrhizae is not clear to us. But since the majority of the plants supplied with mycorrhizae grow on soils rich in decayed material, where saprophytic fungi are the dangerous competitors of the higher plants in the use of nitrogenous and mineral substances, many authors suppose that the fungous mycelium entering into the composition of mycorrhizae is used by the host as an intermediary in the task of getting nitrogenous and mineral substances. The host plant, usually supplies these fungi with non-nitrogenous compounds. From this point of view mycorrhizae may be considered as examples of symbiosis between fungi and higher plants. The

absence of root hairs in mycotrophic plants is counted as one of the proofs in support of this theory.

From isolations in pure cultures it has been found that the mycorrhizal fungi belong to the group of root parasites, many of them being unable to exist without the host plant. The recent detailed investigations by Melin have shown that many of the edible mushrooms, for example, birch, aspen mushrooms[1] and others, the very names of which indicate their adaptation to certain trees, likewise are mycorrhizal fungi, and that they can be cultivated only by growing with them seedlings of the proper species of trees. On the contrary, it is possible to grow mycotrophic trees and other plants without the fungus component. Therefore, some consider mycorrhizae to be in a condition of balanced parasitism. In many of their characteristics, mycorrhizae are similar to the tubercles of leguminous plants; hence, the opinion has frequently been expressed that with their help fixation of molecular nitrogen of the atmosphere likewise may take place. These suppositions, however, have received no exact experimental substantiation.

75. Translocation of Organic Substances. The Deposition of Food Reserves.—The organic substances produced in the leaf by photosynthesis and those stored as food reserves in seeds and other storage organs must be translocated to the place of their consumption. We have already discussed one of the first prerequisites for their translocation, namely, their hydrolysis into more movable compounds. The more complex transformations which fats and proteins undergo also has been noted. The substances most used by a plant and most easily translocated in its body are the sugars and amino acids, especially asparagine.

The initial synthesis of all these compounds takes place in the leaves, and it is not difficult to show by simple experiments that this is followed by an uninterrupted movement of these substances from the leaves. It is possible to demonstate this by Sachs' dry-weight method by which one may also study the accumulation of substance during the process of photosynthesis (Art. 2). If one-half of a leaf is cut off and dried in the evening, and the other half in the morning, it will be found that in the latter case the weight per unit leaf area will be considerably less. Thus, Sachs noted that a square decimeter of leaf area of the sunflower weighed 0.8 g.

[1] *Boletus scaber* and *B. aurantiacus.*

in the evening, but in the morning only 0.7 g. In this case, the leaf lost during the night 0.1 g. of organic substance per square decimeter, or about 0.2 g. per leaf, if it is assumed that the average sunflower leaf has an area of 2 sq. dm. Whence, it is seen that early in the morning leaves of plants are considerably poorer in organic substances than in the evening. This translocation from the leaves, of course, goes on not only during the night but also during the day apparently with an almost equal rapidity. But, during the day, the outgo is overbalanced by the production of substance in the course of synthesis. This may be shown if the increase in weight of the halves of the leaves remaining on the plant be compared with the increase in weight of leaves cut from the plant but placed under such conditions (sufficient water supply, moist atmosphere, etc.), that synthesis will proceed uninterruptedly. It will be found that the cut off leaves accumulate considerably more dry matter, sometimes twice as much as those remaining on the plant. This difference indicates the quantity of substances that have passed from the leaf into the stem during the day.

In the autumn, when the time of leaf abscission approaches, besides this uninterrupted outflow of assimilants, gradually diminishing with the decrease in synthetic activity, the movement from the leaves of another type of compounds more firmly bound within them, is observed. Evidently, this is connected with the gradual aging of the cells and the breaking down of a part of their integral substances. Falling leaves as a rule are considerably poorer in nitrogen, phosphorus, and potassium than those still active. Calcium salts, on the contrary, remain in fallen leaves.

A translocation of substances of similar nature takes place in annual plants, in which at the time of maturation of the seeds all other parts of the plant, leaves, stems, and roots, are gradually emptied of food substances and finally die. Water-culture experiments with corn, for example, have shown that after flowering the plants may be transferred to distilled water and still will give normal development of seeds. It is apparent that all those compounds of nitrogen, phosphorus, sulphur, and other elements, which are stored in the seeds, are taken from the vegetative parts. At the end of development, even the carbon compounds are taken in greater amounts from the older parts than they are synthesized. It has been observed that the average dry weight of a wheat specimen, taken from the field, does not increase between the time of

flowering and the maturing of the grain, notwithstanding the rapid increase in weight of the grain, which in the end contains almost two-fifths of the total dry matter; hence, it is apparent that the weight of the remaining organs of the plant must have decreased by the same amount.

The substances that are removed from the leaves are distributed throughout the plant. Part of them are utilized by the growing regions, the young developing stems and leaves and the tips of roots. Another fraction is directed into the ripening fruits and seeds, or the underground storage organs, or the living tissues of the trunk of the tree, where it is stored as a food reserve. The storage processes are exactly the reverse of those which take place at germination, from the incoming sugars and amino acids there are formed polysaccharides, mainly starch, fats, and reserve proteins. The chemistry of the synthetic processes is not nearly so well known as the chemistry of hydrolysis of these reserve substances. It should be noted that in all of these processes water is given off. Hence, in general, the maturation of seeds and the ripening of the wood in the fall in preparation for dormancy, proceed more rapidly in dry weather, when water is being continually removed. An important rôle in synthesis during food storage is apparently played by enzymes, the action of which, as we saw in Art. 68, is reversible. One may, therefore, suppose that the synthesis of polysaccharides, fats, and proteins is brought about by the activity of the same carbohydrases, lipases, and proteases which bring about their hydrolysis at germination.

Nutritive substances move largely through the sieve tubes and, in general the phloem parts of the conducting vessels. The most evident proof of this has been furnished by ringing experiments with branches or even whole tree trunks. In woody plants there is a solid ring of xylem, through which water moves, and on the outside of it a solid ring of phloem, composing the conducting part of the bark. If a branch of willow is ringed by cutting and removing the bark 1 to 2 cm. in width, and then placed in water, so that the ringed portion will remain in damp air, it will be seen that the adventitious roots develop considerably better and in greater number above the ringed portion (Fig. 98), while the control branch will have most of its roots at the very base of the branch. If a branch is ringed on the tree, the bark at the upper edge of the cut will begin to thicken more, forming a ringlike

swelling or callus (Fig. 99), while no annual rings will be formed below the cut. Finally, if the trunk of a tree is ringed, it will remain fresh for a long period, because the water rises through the wood, which is not injured by the ringing. But, in the end, unless this ring heals over, the tree will die, since the ring has broken the path of movement of nutritive substances to the roots, the activity of which will soon stop. It is worth noting that in

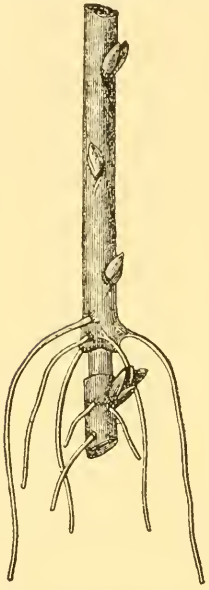


Fig. 98.— A ringed twig of willow showing root development primarily above the ringed portion.

Fig. 99.—A branch showing development of callus at the upper edge of the ringed region (*after Brown*).

plants having sieve tubes, not only near the outside, but also on the inside of the xylem ring (*Oleander*), experiments with ringing have given much less definite results, since the translocation of organic substances along the inner phloem will continue after the cutting of the bark.

Ringing experiments, first conducted by Hales in the seventeenth century, have led to the established view that substances move along two paths in a plant, an ascending and a descending one. The upward flow proceeds through the wood and carries with it inorganic salts, which are obtained by the roots from the soil. The downward flow takes place through the bark and moves with it

organic nutritive substances manufactured in the leaves. On the whole, this opinion remains established at the present time, with the single modification that the flow of plastic substances from the leaves is not always downward. It is directed not only down, into the roots, but also up, to the growing tip of the stem and to the developing branches and fruits.

Moreover, the ascending flow may carry with it organic nutritive substances as well. This usually takes place in the spring at the time of sap movement, when the accumulated reserve substances of the wood and the bark of both the roots and the stem rapidly dissolve and enter in large quantities the vessels of the wood, through which they move upward to the opening buds. The impelling force is the root pressure, which is especially strong at this time of the year. (Arts. 43 and 44.)

Besides the sieve tubes, the parenchyma elements of the phloem also take part in the translocation of organic substances, especially in herbaceous plants. Translocation through the parenchyma, however, must proceed considerably slower than through the tracheal tubes. It plays a considerable rôle only in translocation over short distances similar to the movement of water in this tissue (Arts. 60 and 61).

The mechanism of the translocation of organic substances through the plant is as yet very little understood. It is commonly supposed that the conspicuous rôle here is played by the phenomenon of diffusion, which forces substances to flow in the direction of lower concentration. In the germinating seeds, for example, the greatest concentration of sugar is in the endosperm, where starch is dissolved, and the least in the embryo, where sugar is used. Sugar, therefore, will flow from the endosperm into the embryo. Diffusion processes, however, proceed too slowly to permit one to explain the translocation of organic substances by this mechanism, and still less their accumulation, as in the case of soluble carbohydrates in bulbs or the roots of the sugar beet. Therefore, the laws of diffusion alone are not sufficient to explain the causes of the translocation of organic substances, and many authors suppose that an important rôle is played by the movement of the protoplasm in the conducting cells, which promotes the rapid translocation of these substances. The movement of protoplasm in greatly elongated cells, as for instance in the sieve tubes, may be of especial importance.

CHAPTER IX

RESPIRATION AND FERMENTATION

76. The Rôle and Nature of Respiration.—Heretofore, the metabolism in plants has been considered as a process through which complex organic reserve substances decompose into their elementary constituents. These simple parts are then again formed into complex compounds of the protoplasm. No consideration has been given to the highly important fact that in the second synthetic stage of metabolism a considerable portion of the substances does not serve in the construction of new living matter, but, on the contrary, undergoes a still further disintegration and leaves the organism as end products of oxidation—carbon dioxide and water. This loss of substance is especially evident when seeds are germinated in darkness. By his classical investigations, Boussingault (1868) showed that seeds may lose in this manner over half of their weight. In one of his experiments, grains of wheat before germination weighed 1.665 g. but the seedlings developing from them only 0.712 g., thus showing a loss of 57 per cent. Again, seeds weighing 2.237 g. produced seedlings weighing only 1.076 g., having lost 52 per cent of the original weight.

This loss of substance is not equally distributed between the different constituents of the seed. While the quantity of carbon, hydrogen, and oxygen is continually decreasing, that of nitrogen and of the mineral substances usually remains constant; hence, the conclusion may be drawn that only substances which contain no nitrogen decrease in amount, especially the carbohydrates. This is shown diagrammatically by Prianishnikov (Fig. 100). It will be noted from this figure that in germinating vetch seeds the quantity of nitrogenous substances, in spite of the disintegrations of proteins, remained the same, owing to the accumulation of amino acids and asparagine. The amount of carbohydrates, however, decreased rapidly, since the soluble carbohydrates formed in hydrolysis of starch are used only in small amounts for the construction of cell walls. The greater part disappears.

This loss is due to respiration, which accompanies germination. Respiration is essentially the oxidation of sugars to the end products, carbon dioxide and water. It may be expressed by the following general formula:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O.$$

Considering its end products, this reaction is the reverse of photosynthesis, which has been represented in Art. 3 as follows:

$$6CO_2 + 6H_2O \rightarrow C_6H_{12}O_6 + 6O_2.$$

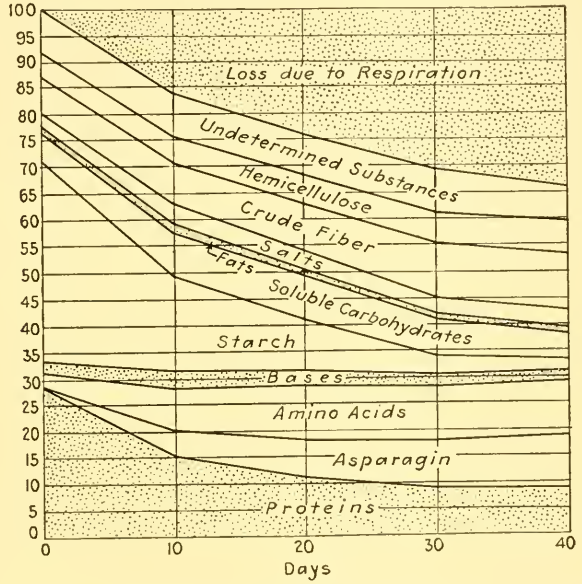


FIG. 100.—The transformation of reserve substances during the germination of vetch seeds (*diagram after Prianishnikov*).

Respiration is also an opposite process to that of photosynthesis in respect to the energy involved. It has been shown that in photosynthesis solar energy is utilized in the construction of carbohydrates. During physiological combustion of carbohydrates, on the contrary, energy is liberated at a rate of 674 Cal. per gram-molecule (180 g.) of glucose used.

This liberation of energy is the essential feature of the respiratory process. For all its vital activities, growth, movement, and

the various endothermic reactions connected with synthetic processes, the plant requires energy, just as do engines. This energy is secured through the combustion of carbohydrates. It differs, however, from the burning of fuel in a furnace, since in protoplasm the energy of oxidation passes into other forms of chemical energy and is emitted as heat energy only after having been utilized and changed. These internal transformations of energy which accompany respiration are, however, very little understood; hence, the total quantity of energy obtained through respiration is still measured by its thermal effect.

Respiration has always been considered as being closely connected with life. This is but natural, since life is subject to a continuous liberation of energy obtained through respiration. Therefore, the investigation of the respiratory process is one of the central problems of general physiology. And the deepest analysis and furthest penetration into the chemical and dynamic nature of this phenomenon promises us a clearer understanding of the complex processes called life.

In both animal and plant organisms, respiration is essentially of the same nature. It consists in obtaining energy for the cells during the combustion of carbohydrates. Usually, the animal organism consists of a massive body to some parts of which gaseous oxygen cannot easily penetrate; hence, in order to provide all cells with oxygen a special complex mechanism is required, which consists of a blood-circulating system with its conveyors of bound oxygen, the red blood corpuscles, and respiratory organs, where the blood can be saturated with oxygen and freed from the accumulated carbon dioxide. The complexity of these essential, yet adventitious, organs has obscured the true nature of respiration. Even as late as the beginning of the nineteenth century, the opinion still prevailed that respiratory movements and blood circulation were the main features of respiration, and, consequently, the existence of respiration in plants was denied. Much effort was necessary to introduce into science the truth that respiration proceeds neither in the lungs nor in the blood, but in each living cell.

The external conditions of respiration are very much simpler in plants than in animals. Owing to the development of a comparatively large surface, as a result of gaseous nutrition, the oxygen of the air comes in direct contact with each cell of the plant body.

The oxidation processes therefore are not obscured by any external features. Naturally then, the attention of botanists has been continually directed to the study of the nature of this phenomenon, and it was in plant physiology that the question of the internal chemistry of respiration has received special consideration.

77. Methods of Studying Respiration. Intensity and Localization of Respiration in Different Plants.—The methods of studying respiration in plants are essentially methods of determining the exchange of gases between the plant body and the atmosphere. Fundamentally, the method is identical with the study of the gas exchange during photosynthesis. Generally, it consists in placing a respiring plant or part of it in a hermetically sealed container, which is provided with lateral tubes through which from time to time samples of gas can be taken for analysis by the same apparatus which is used in the study of photosynthesis. Formerly, the eudiometric method, described in Art. 2, was employed also. All experiments with green plants or those which are able to become green must, of course, be conducted in darkness, lest the opposite process of absorption of carbon dioxide should superimpose itself on respiration.

Often, the study of respiration is confined only to the determination of the liberated carbon dioxide, which, of course, is more readily made than is a complete gas analysis. For this purpose, the respiring parts of a plant are placed in a container through which is sucked an air current that has been cleared of carbon dioxide. Having passed over the plant, the air current leaves the carbon dioxide, caught on its way, in a special absorbing apparatus. This may be either a U-shaped tube containing potassium hydroxide or soda lime, or one of the various vessels containing potash. The quantity of carbon dioxide formed per unit of time is estimated by the increase in weight of the apparatus. Previous to entering into the apparatus the current of air must be dried. Frequently, a titrable solution of barium hydroxide is used as an absorber of CO_2, with Pettenkoffer's tubes used as a convenient equipment (Fig. 101), through which an air current passes in the form of fine bubbles which discharge to the barium hydroxide their carbon dioxide. The quantity of the latter is determined by the difference between the initial and final titration figures of the solution.

There is considerable variation between the intensity of respira-

tion in different plants and in different parts of the same plant. Respiration is closely connected with growth, and, therefore the greater the energy of the latter, the higher is the respiratory rate. Rapidly growing organs, whose cells are rich in protoplasm, often respire more intensely than do animals. Thus man, for instance, produces in 24 hr. a quantity of carbon dioxide weighing about 1 to 2 per cent of his body. The respiration of germinating seeds at a temperature of 37° C. is of the same intensity. Still greater is the respiration of molds, which produce as much as 6 or even 10 per cent of carbon dioxide in 24 hr. per unit weight.

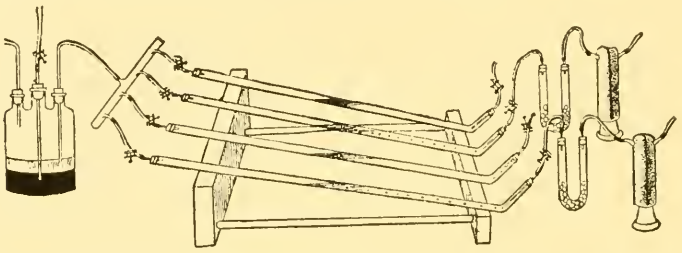


FIG. 101.—Pettenkoffer's apparatus for quantitative determination of carbon dioxide eliminated in respiration. The germinating seeds are placed in U-shaped tubes connected with cylinders, containing soda lime and absorbing CO_2 of the air. The air current proceeds from right to left (*after Palladin*).

The following table gives an idea of the quantity of carbon dioxide which is liberated by various plant organs during 24 hr. at room temperature per 1 g. of dry weight.

	Cubic Centimeters
Full-grown leaves	12 to 24
Growing rootlets	40 to 80
Buds of trees	35 to 70
Germinating seeds	60 to 120
Growing mycelia of fungi	270 to 1,800

The respiration of flowers, especially when unfolding, is as intense as that of germinating seeds.

The object of respiration in plants is the liberation of energy for use in vital processes. Because of their enormous surface and a comparatively low rate of respiration, it is impossible for plants to maintain a temperature at a higher level than that of the surrounding air. But, when a considerable quantity of intensely respiring plant material, such as germinating seeds, flowers, or

leaves, are put into an insulated container, for instance a Dewar's flask (Fig. 102), a rise in temperature of 30 to 40 or even 50° C. may be observed. This extreme heat may eventually result in death of the plant material. Still more accentuated is the rise of temperature during the respiration of fungi and bacteria. On damp hay, for example, there develops rapidly such a rich flora of microbes, that it soon becomes very warm and may "heat," if it is not scattered. A considerable rise in temperature may be observed also in some large flowers. The flowers of *Victoria regia*, for instance, may have a temperature 12° C. higher than the surrounding air. In the inflorescences of *Arum*, *Colocasia*, and other aroids, this difference can be as high as 30° C.

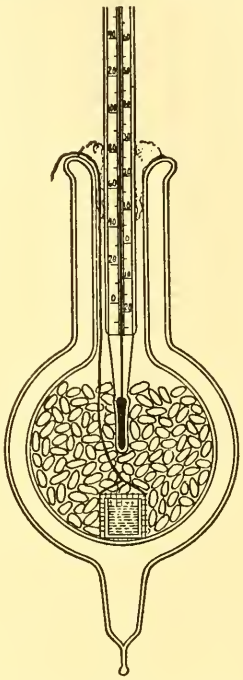


Fig. 102. — Flask for the study of increase in temperature induced by respiration of germinating seeds (*after Ganong*).

Part of the energy liberated during respiration may take the form of light. Phosphorescence, however, is observed only in the lower plants, the fungi and bacteria. Their activity explains the phosphorescence of rotted wood, sea fish, meat, etc. Phosphorescence, though, is not always the result of respiration. It may also be due simply to oxidation by the air of special phosphorescing bodies of protein character produced by the organism.

78. Substances Consumed during Respiration. The Respiratory Coefficient.— The fundamental respiratory materials, as has been seen, are the sugars, especially glucose. From the general equation of respiration $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$, it is clear that the volume of the gases exchanged, namely, the oxygen absorbed and the carbon dioxide liberated, must be equal. A plant placed in a closed container therefore, does not alter the volume of air, though it changes its composition, substituting carbon dioxide for oxygen. In the more typical cases, the ratio of carbon dioxide to oxygen in respiration, $\frac{CO_2}{O}$, called the "respiratory coefficient,"

is equal to unity. The same quantitative relationship exists between the interchanging gases in photosynthesis, where oxygen is liberated and carbon dioxide is absorbed.

There are many deviations from this general rule, as very often the respiratory coefficient is less than unity. Sometimes, it is more. Various causes may be responsible for these differences. Besides the end products of oxidation, CO_2 and H_2O, frequently there are formed but partly oxidized compounds, such as oxalic acid, $C_2O_4H_2$, tartaric acid, $C_4O_6H_6$, etc., which contain more oxygen than carbohydrates normally do. When these substances are produced, part of the absorbed oxygen remains in the plant, and the ratio of $\frac{CO_2}{O_2}$ is less than unity. On the other hand, when oxygen is deficient, as in the respiration of bulky organs or seeds immersed in water, normal respiration may be accompanied by alcoholic fermentation, resulting in the liberation of carbon dioxide without absorption of oxygen from the air. In such cases, the respiratory coefficient will be greater than unity.

The respiratory coefficient may diverge considerably from unity in cases when the respiratory material is not sugar, but some other substance which contains different quantities of oxygen and hydrogen than sugar does. When the substance is richer in hydrogen, as for instance, a fat or a protein, part of the oxygen will be utilized in the oxidation not only of the carbon but also of hydrogen, and the respiratory coefficient will fall as low as 0.7 to 0.8. Thus, for instance, in the oxidation of stearic acid, the reaction proceeds according to the formula

$$C_{18}H_{36}O_2 + 26O_2 = 18CO_2 + 18H_2O,$$

and the ratio of $\frac{CO_2}{O_2}$ will be $\frac{18}{26}$ or 0.69.

In combustion of substances containing more oxygen than do carbohydrates, such as the organic acids, the respiratory coefficient, on the contrary, will be greater than unity. Thus, in combustion of oxalic acid, according to the equation,

$$2C_2O_4H_2 + O_2 = 4CO_2 + 2H_2O,$$

the respiratory coefficient is 4; hence, it is quite clear that the lower

the respiratory coefficient, the higher is the heat liberation during combustion, and vice versa. Proteins and fats exhibit therefore a high heat equivalent, organic acids a low one.

Variations in the heat equivalent in dependence on the food material supplied may be most readily observed in molds which possess scarcely any reserve substances of their own, but are endowed with a very high intensity of respiration. When such fungi are grown on carbohydrates, the $\frac{CO_2}{O_2}$ ratio is almost equal to 1; when cultivated on fats, it falls to 0.7; and on acids, it rises to 2 to 3. But if these organisms are supplied with a mixture of sugar and other substances, the coefficient will be at first close to 1, until the sugar is spent. Then the combustion of the other substances will begin with a consequent change in the ratio. It is interesting to note that even in oily seeds the coefficient may be increased until it approaches 1, if sugar is supplied from without. This shows clearly that sugar is the chief respiratory material. Kostytschev is even of the opinion that sugar is the only directly combustible material, the other substances, such as proteins and fats, undergoing transformation into sugar previous to combustion.

The predominant rôle of the sugars in nutrition, as well as in respiration, is moreover suggested by the interesting fact that molds develop considerably slower on fats than on sugars. The reason of this is that fats must previously undergo a complex transformation, while sugars are utilized directly. The same is indicated by the transformation of fats into sugars during the germination of oily seeds (Art. 69).

79. Respiration at the Expense of Mineral Substances. Nitrification, Sulphur and Iron Bacteria.—The general rule of preference of sugar in respiration to all other materials does not apply to certain groups of bacteria using inorganic substances for respiration. In these bacteria one finds a peculiar process of carbon assimilation, which proceeds not at the expense of the radiant energy but as a result of the oxidation energy of such substances as ammonia, hydrogen sulphide, etc. This is a process of chemical synthesis (Art. 8). Here belong in the first place the nitrification bacteria, which live in the soil and convert ammonia, formed by the decomposition of organic refuse, into salts of nitrous and nitric acid.

Winogradsky's classical investigations show that these oxidations are achieved by two kinds of bacteria; the first, called *Nitrosomonas*, having the form of small mobile cocci oxidize ammonia to nitrous acid, according to the equation, $2NH_3 + 3O_2 = 2HNO_2 + 2H_2O + 158$ cal.; the others, called *Nitrobacter*, and of the form of immobile short rods (Fig. 103), oxidize nitrous acid, produced by the first group, into nitric acid, according to the equation, $2HNO_2 + O_2 = 2HNO_3 + 38$ cal.

One will note that the second process yields considerably less energy (calories). This may be collated with the fact that *Nitrosomonas* needs on the average for the assimilation of 1 atom of carbon the oxidation of 35 molecules of ammonia, while *Nitrobacter* utilizes for the same quantity of carbon 135 molecules of nitrous acid.

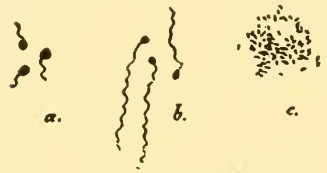


FIG. 103.—Nitrobacter.

Availing themselves of the oxidation of nitrogen compounds as a source of energy and securing carbon from carbon dioxide of the air, the nitrifying bacteria not only require no organic compounds but are incapable of transforming them. Furthermore, organic substances, among them the sugars, which are necessary to all other organisms, are poisonous to them. Yet, in spite of all the peculiarities of their metabolism, these bacteria are nevertheless made of materials similar to those of other plants. Protein, in particular, may be found in these organisms, though the question, whether carbohydrates also take part in the building up of their bodies, remains as yet unsettled.

The nitrifying bacteria have a very important rôle in the general cycle of nitrogen in nature. They are present in all soils save marshy ones. Moreover, all the nitrates existing under natural conditions are exclusively the result of their activity. A very peculiar feature of these organisms is the strict harmony in the work of both groups. The nitrite- and nitrate-forming bacteria are so closely united, that Winogradsky found it difficult to separate them.

The physiology of another group of organisms utilizing inorganic substances during respiration, *viz.*, the sulphur bacteria, has also been investigated through the work of Winogradsky. These organisms were referred to in Art. 25, when the sulphur cycle

in nature was considered. The oxidation of hydrogen sulphide proceeds according to the following equation: $H_2S + 2O_2 = H_2SO_4 + 115$ cal.

As in the case of nitrification, so here the heat energy given in calories is utilized by the bacteria, not only to obtain the necessary energy for their vital processes, but also for the decomposition of carbon dioxide. Consequently, these bacteria do not need organic substances. Contrary to the nitrification bacteria, the sulphur organisms can grow without detriment in the presence of considerable amounts of organic substances in the nutrient medium.

The sulphur bacteria represent a rather varied group of organisms. They include single-celled as well as threadlike forms. Some of them contain a bright reddish purple pigment, called "bacteriopurpurin." Hence, they have received the name of purple bacteria. Engelmann has advanced the opinion that this pigment is a substitute for chlorophyll and is effective in the decomposing of carbon dioxide. In fact, light, which generally suppresses the development of bacteria, has a favorable influence upon the purple bacteria. Possibly the oxygen eliminated in the decomposition of CO_2 facilitates the oxidation of hydrogen sulphide by these organisms. This permits them to live in water which is saturated with hydrogen sulphide and poor in oxygen, and to a degree excludes the possibility of development of other microbes.

Besides the nitrifying and sulphur bacteria there exist large numbers of microorganisms whose respiration proceeds at the expense of mineral substances. Of particular significance are the iron bacteria, first described by Winogradsky, which convert ferrous into ferric salts. The most important of these are the threadlike *Leptothrix*, *Crenothrix*, and other related forms. They play an important rôle in nature. The so-called bog ores, immense accumulations of oxidized iron at the bottom of bogs and lakes, are the results of their activity. Some of these bacteria are able also to transform organic substances. They are not so absolutely autothropic as the nitrifying organisms. Besides ferrous salts, many of them also can oxidize manganese compounds.

In nature, especially in bogs, there are widely distributed bacteria which can obtain energy through the oxidation of marsh gas (methane) or of hydrogen. The chemistry of this oxidation and the metabolism of these organisms are at present very little

understood. They appear to belong to a group of facultative saprophytes, that is, they can develop on organic substances. The oxidation of methane and hydrogen is of great advantage from a kinetic point of view, since in the combustion of hydrogen a great quantity of energy is liberated. These bacteria are able to utilize this energy for the decomposition of CO_2, but only in the presence of a considerable amount of ferrous salts.

80. Anaërobic Respiration and Alcoholic Fermentation.—Normal respiration represents an oxidation process which continues during the uninterrupted absorption of oxygen of the air. When a plant is transferred into an oxygen-free atmosphere, respiration must inevitably cease. Together with it are discontinued the processes directly connected with the liberation of energy obtained through respiration, as, for instance, growth and protoplasmic as well as organic movements. When the plant is permanently deprived of oxygen, it will perish, since for its support, life requires a continuous supply of energy.

In the absence of free oxygen, death of a plant does not occur immediately, since life may be supported in the cells for some time by anaërobic respiration. As in both normal and anaërobic respiration carbon dioxide is eliminated, oxidation processes take place in either case. In the absence of air, however, the necessary oxygen is taken from various compounds, as, for instance, water and the hydroxide groups of oxidized sugar molecules; hence oxidation during anaërobic respiration is accompanied by reduction.

Carbon dioxide is the most oxidized product of anaërobic respiration, while alcohol is a highly reduced product. The general reaction may be represented by the following equation:

$$C_6H_{12}O_6 = 2CO_2 + 2C_2H_5OH + 25 \text{ Cal.}$$

When compared with the amount of free energy released by complete oxidation of a molecule of glucose, *viz.*, 674 Cal., this quantity is very small. It is but natural, therefore, that the energy of anaërobic respiration is not sufficient to maintain all the processes which usually are supported through ordinary respiration.

Besides the low energy value, anaërobic respiration has the other disadvantage of accumulating alcohol, a substance known to possess poisonous properties. Consequently, in the absence of oxygen, a plant perishes not merely from an excessively low supply

of energy, but likewise from self-poisoning. In this respect the experiments by Nabokich are very instructive. He placed germinating pea and sunflower seeds in a large vessel containing water, and by continued evacuation removed the last traces of air. The seeds not only remained alive for several weeks, but even showed some growth. This is explained by the fact that the poisonous products of anaërobic respiration were leached out by the water. In a gaseous oxygen-free medium the same seeds exhibit no signs of growth. They die after 3 to 5 days.

The usual material for anaërobic as well as normal respiration is sugar. Other substances, as for example, glycerine, mannite, tartaric and quinic acids, peptone, etc., may also be utilized. It appears, however, that the breaking down of these substances is preceded by their conversion into sugar, or at least into closely related substances.

Anaërobic respiration, which in the higher plants is but a temporary substitute for normal respiration, is in certain microorganisms the fundamental process of securing the necessary energy. Yeast is a most conspicuous example of such microorganisms. Its anaërobic respiration is called "alcoholic fermentation."

Alcoholic fermentation has been known to mankind since time immemorable. It is applied not only in the preparation of beverages, where the alcohol produced by yeast is used, but also in bread making, where the counterpart of fermentation, the elimination of carbon dioxide which raises the dough, is utilized. For a long time, the nature of this process remained mysterious. It was often ascribed to a purely chemical "autodecomposition" of sugar. By his classical investigations, Pasteur (1860) succeeded in ascertaining correctly the biological nature of alcoholic fermentation and in proving that fermentation is the manifestation of vital activities of yeast.

Yeast belongs to the genus *Saccharomyces*, related to the *Ascomycetes*. These are single-celled organisms which multiply rapidly by budding, and sometimes form fragile chains. Under special conditions there are produced within each cell four spores (Fig. 104). A great number of various kinds of yeast are known. They differ from each other physiologically as well as morphologically; for instance, by the form and size of their cells. All forms produce typical alcoholic fermentation and give rise to different byproducts,

which influence in various ways the quality and the flavor of the beverages obtained. Some species, as for instance, beer-yeast, *Saccharomyces cerevisiae*, consist of many varieties or races, each of which gives the beer a taste of its own. Almost every large brewery, particularly in Germany, uses its own race of yeast and has a special microbiological laboratory for maintaining its purity.

Only definite substances, carbohydrates with 3, 6, and 9 carbon atoms (trioses, hexoses, and nonoses) are fermentable; di- and polysaccharides may be fermented only after previous hydrolysis. But since yeast possesses active invertase and maltase, but no diastase, it fails to ferment starch directly, which must be pre-

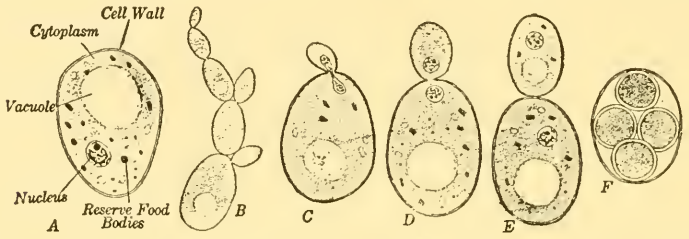


FIG. 104.—Yeast. *A*, structure of cell; *B*, chain of budding yeast; *C*, *D*, *E*, successive stages of budding; *F*, spores (*after Smith et al.*).

viously changed into sugar. Hence, in the preparation of beer one cannot start with barley before it has germinated, since it is very poor in diastase; but must use malt in which the quantity of diastase has considerably increased during germination. Moreover, before adding yeast, it is necessary to "cook" the beer mash, thus changing the remaining starch into maltose at 60 to 70°, the optimal temperature for the action of diastase.

Not all hexoses can be fermented by yeast. As in respiration, so in fermentation only the *d*-glucose, *d*-fructose, *d*-mannose and *d*-galactose are fermentable, the last two sugars being utilized considerably slower than the others. Yeast can ferment rather pure solutions of sugars, but like other saprophytic organisms, it requires for its full development all the necessary mineral substances, and a ready source of nitrogen, preferably in the form of peptones or amino acids. Ammonia is also suitable for the synthesis of proteins by yeast. When amino acids are present in abundance in the fermenting liquid, they are deaminized and often

are reduced. As a result of this process, they produce the higher homologues of alcohol, the so-called fusel oils, while the ammonia formed goes into the building of protein molecules. By introducing ammonia salts into the solution, the fermentation of the amino acids can be markedly suppressed.

The process of deamination may be expressed by the following equation:

$$R \cdot CHNH_2 \cdot COOH + H_2O = RCH_2OH + NH_2 + CO_2.$$

In reality, the reaction does not consist in the simple addition of water but, like the process of alcoholic fermentation (Art. 81), it represents a combination of oxidation and reduction. The chief constituents of fusel oil are amyl and isoamyl alcohol.

Alcoholic fermentation may be produced also by certain fungi of the mucor group. Developing upon sugar solutions in the absence of oxygen, these fungi, instead of forming a hyphal mycelium, form individual roundish cells, called "mucor yeast." Fermentation by mucor does not differ in any essentials from that produced by yeast, but it is more sensitive to the presence of large amounts of alcohol and stops as soon as the latter has reached 2 to 3 per cent. True yeast, on the contrary, is very tolerant of ethyl alcohol and can continue its activity till 14 to 16 per cent of it has accumulated in the liquid. Because of this endurance, yeast utilizes fermentation not only as a source of energy but also as a means in competition with other organisms. Grape juice, for instance, will readily spoil before fermentation, due to the activity of various organisms, while wine, having undergone fermentation, is a stable product. The accumulated alcohol prevents the development of the majority of microorganisms, with the exception, however, of the acetic-acid bacteria (Art. 82). To the latter, alcohol is as much of a specific material of respiration as sugar is to the majority of other organisms.

A higher concentration of alcohol than 14 to 16 per cent cannot be obtained by natural fermentation; hence, in order to prepare stronger beverages, methods of distilling fermented liquids are used.

81. Enzymes and Their Chemistry in Alcoholic Fermentation.—Considered from a purely practical as well as from a theoretical point of view, alcoholic fermentation is a process of extreme importance. It has been seen already that it may substitute normal

respiration. According to a well-known statement made by Pasteur, who first demonstrated the existence of anaërobic organisms, it is "life without oxygen."[1] At the same time, its chemistry is simpler than that of splitting of the respiratory material in aërobic respiration accompanied by oxidation. The study of the chemistry of alcoholic respiration has attracted the attention of investigators, for a long time, but only recently has a successful solution of this question been found.

Previously, fermentation was considered to be the result of general metabolism within the yeast cell. The organism was supposed to absorb sugar and after a series of transformations of the sugar molecule, to eliminate alcohol and carbon dioxide. A number of investigations have shown, however, that fermentation and the vital activity of yeast are two different processes. Thus, if yeast is supplied with pure sugar in the absence of nitrogen and mineral substances, it will ferment the sugar, but will not multiply. On the other hand, fermentation in the presence of oxygen proceeds somewhat more slowly, but oxygen exerts on the multiplication of yeast cells a pronounced favorable influence. In order to obtain more alcohol it is expedient, therefore, to limit the access of oxygen, while for obtaining the largest possible quantity of yeast, as in the manufacture of pressed yeast, it is more profitable to give the liquid abundant air.

The experiments by Buchner have supplied conclusive evidence that fermentation may take place apart from the manifestation of vital activities of the yeast cell. Having ground up yeast with sand and diatomaceous earth, he subjected the mass under a hydraulic press to 300 to 400 atmospheres pressure and succeeded in obtaining a transparent juice which contained no living cells, but nevertheless caused sugar to ferment. The active substance in this juice could be precipitated by acetone or alcohol, and redissolved without losing its capacity to produce fermentation. This substance is classified as an enzyme and is called "zymase."

The discovery of zymase created an epoch in the study of fermentation and enzymatic reactions in general. For the first time an enzyme was obtained which produced not merely a simple hydrolysis but a very complex process till then considered to be the result of a complicated metabolism. In due time there were discovered simpler methods of obtaining zymase, for example, by

[1] This of course does not mean the absolute absence of oxygen.

treating yeast cells with acetone or by carefully drying them and subsequently extracting with water, which, of course, considerably facilitated investigation of the enzyme. Further studies have shown that zymase is not a single enzyme, but a complex of several enzymes. This agrees with the fact that alcoholic fermentation is a very complex process proceeding through several separate phases.

Not all of the phases of alcoholic fermentation have been definitely determined as yet, but recent investigators, notably Neuberg and his co-workers, and Kostytschev, have brought us nearer to the solution of this question. According to these investigations, the most important intermediate products in fermentation are pyruvic acid, $CH_3 \cdot CO \cdot COOH$ and acetaldehyde, $CH_3 \cdot COH$. In their details the mechanisms as proposed by Kostytschev and by Neuberg differ considerably.

Kostytschev supposes that the first phase comprises the elimination of hydrogen from sugar, attended by the formation of pyruvic acid $C_6H_{12}O_6 = 2CH_3 \cdot CO \cdot COOH + 4H$, the hydrogen being bound by special hydrogen acceptors. Then under the influence of carboxylase, a special enzyme splitting off CO_2, pyruvic acid is converted into aldehyde and carbon dioxide, $CH_3 \cdot CO \cdot COOH = CH_3 \cdot COH + CO_2$. Finally, the formed aldehyde is reduced by the previously bound hydrogen to alcohol,

$$CH_3 \cdot COH + 2H = CH_3CH_2OH.$$

Kostytschev indicates that the first part of his theory is of a purely hypothetical character.

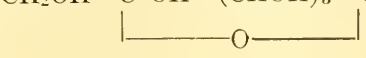
In Neuberg's proposed mechanism a great rôle is played by the Cannizzaro reaction, *i.e.*, the conversion of 2 molecules of aldehyde, with addition of water, into 1 molecule of acid and 1 molecule of alcohol, through mutual interchange of oxygen and hydrogen. Neuberg considers methylglyoxal, $CH_3 \cdot CO \cdot COH$, as the first product of fermentation, which then by way of transmutation under the influence of a special enzyme, mutase, produces glycerine and pyruvic acid. The latter splits into CO_2 and acetaldehyde, $CH_3 \cdot COH$, which in its turn produces pyruvic acid and alcohol.

Besides Kostytschev's and Neuberg's hypotheses, there have been proposed also other ideas. Both Buchner and Palladin, for

instance, consider lactic acid as one of the intermediate products. These various theories will not be discussed in detail here, since none of them give a satisfactory explanation of the internal chemical processes of fermentation and none can be considered as finally proved. Certain fundamental ideas common to all of the theories need to be emphasized, however. In the first place, there is no doubt that in one of the first phases of fermentation, glucose, a six-atomic carbon chain, breaks into two three-atomic segments. This explains why only trioses, hexoses, and nonoses are fermentable, but no pentoses or heptoses. A very important factor also, is the process of reduction in alcoholic fermentation. The capacity of reduction by yeast has been known for a long time. It has been noted, for example, that after the addition of sublimed sulphur to a fermenting liquid, hydrogen sulphide is formed. Methylene blue may be reduced in the same manner to a colorless leuco compound (containing 2 hydrogen atoms more than the dye itself; nitrobenzole, to aniline, etc.). All these reactions indicate that during fermentation activated hydrogen is formed, which through a special reducing enzyme, called reductase, is transferred from the substance containing hydrogen to the hydrogen acceptor, such as sulphur and the other substances mentioned. In the normal course of fermentation the hydrogen serves to reduce acetaldehyde.

A very interesting feature is the active part played by phosphoric acid in alcohol fermentation. It has been proven that phosphates considerably accelerate the fermentation of sugar, sometimes ten to twenty times. This is especially the case when fermentation is produced not by yeast but by zymase. The investigations of L. Ivanov (1905) as well as of Harden and Young (1905) have shown that during the first stages of fermentation there is formed a diphosphoric ether of fructose, $C_6H_{10}O_4(Na_2PO_4)_2$, the so-called zymophosphate, a special enzyme, phosphatese taking part in this synthesis. With the further progress of fermentation, the zymophosphate splits up again under the action of the same phosphatese, always producing *d*-fructose, no matter from what fermentable hexose it was formed. The stimulating influence of phosphates on fermentation has not yet been fully explained. It is possible that the transformation of all hexoses into the same form of *d*-fructose may be brought about. The most active sugar taking part in all vital processes is glucose, with a closed chain

structure, the so-called γ-glucose, which may have the following structure:



In the free condition, γ-glucose is very unstable and assumes an inactive form, but it may be isolated in several combinations.

It is interesting to note that in the molecule of sucrose, the glucose is present in active form, and that by inversion first is obtained γ-glucose, which later is transformed into the inactive form. It is possible that this capacity of producing the most active of all hexoses, explains the wide distribution and high importance of sucrose in the plant body, and the animal organism.

No matter what alterations in our ideas of the chemical processes of alcoholic fermentation may take place in the future, it must at any rate be considered as a complex catalytic process caused not by one, but by several enzymes. In the living cell all these enzymes are working in harmony with each other and the process of fermentation goes on as smoothly as if it were a simple reaction. But in a killed cell, or in the expressed sap, the system of enzymatic activity is disturbed and fermentation soon ceases. Hence the total quantity of sugar fermented by various preparations of zymase is always insignificant if compared with the amount fermented by living yeast cells.

82. Acid Fermentation and Its Chemistry.—Besides alcoholic fermentation, there exists a number of other types. The most important of these are lactic, butyric, and acetic fermentation. The first two consist of the breaking down of sugar accompanied by the formation of the corresponding acids, while the third represents a process of oxidation of alcohol by oxygen of the air. It is more akin to respiration than to alcoholic fermentation. Pasteur was the first to investigate the various kinds of fermentation and to establish their biological nature. He isolated in pure cultures the microorganisms by which they are caused.

Lactic-acid fermentation is produced by special bacteria. It has received its name from the chief end product, lactic acid, $CH_3 \cdot CHOH \cdot COOH$. These bacteria fall into several groups. One of these causes purely lactic fermentation, *i.e.*, the splitting

of the sugar molecule into 2 molecules of lactic acid, according to the formula

$$C_6H_{12}O_6 = 2CH_3 \cdot CHOH \cdot COOH.$$

Bacteria producing lactic acid cause the normal souring of milk. Once the sour reaction is initiated, the development of other bacteria ceases, and the coagulation of casein is produced, resulting in curdling of the soured milk. As coagulation proceeds further, the clots of casein contract and whey is separated out. Further accumulation of lactic acid stops the development of the very organisms that cause this fermentation; hence, there never accumulates more than 1 per cent of acid, and part of the sugar does not decompose. But if fermentation is conducted in the presence of calcium carbonate, $CaCO_3$, which neutralizes the acid as soon as it is formed, fermentation of the sugar proceeds to the end. The same hexoses which undergo alcoholic fermentation, as well as maltose and lactose, can be subjected to lactic-acid fermentation. Cane sugar, on the contrary, is not fermented by most lactic-acid bacteria, since they do not produce invertase.

Impure lactic fermentation is a rather complex process. Besides lactic acid, there are formed great quantities of acetic acid, a little ethyl alcohol, carbon dioxide, and hydrogen. Fermentation of this type proceeds best in the presence of air, while the bacteria causing pure lactic fermentation do not need it. Impure fermentation only spoils milk. In order to obtain faultless curdling, therefore, boiled or at least pasteurized milk is used with pure cultures of lactic-acid bacteria. For the preparation of certain food products, such as sauerkraut, pickles, etc., impure lactic fermentation is used. This process is accompanied by rapid elimination of gases, while the accumulating acetic and lactic acids prevent the development of bacteria which cause decay and help to keep the products in good condition. Care must be exercised, however, not to let the air have access to the fermented products, since they are a favorable substrate for molds preferring a sour medium.

Butyric-acid fermentation differs from the lactic-acid type in being anaërobic. Bacteria causing it are not only able to live without atmospheric oxygen, but are even unable to develop in the presence of it. According to their response to oxygen, all microorganisms may be divided into three fundamental types:

obligate aërobic forms, which perish like most higher plants in the absence of oxygen; facultative anaërobes, able to live in the presence of air or without it, and obligate anaërobes, dying when exposed to free oxygen. The butyric-acid bacteria belong to the last group.

Of the several types of butyric-acid bacteria the most characteristic are those belonging to the genus *Clostridium*. They cause milk and butter to turn rancid and are of economic importance in the maturing of cheese. Here belongs also *Clostridium pasteurianum* already considered, which fixes molecular nitrogen. The genus *Clostridium* is characterized by the production of very resistant spores, some of which will resist even boiling for a short time. Hence butyric-acid fermentation may take place in products which previously have been boiled. Insufficiently boiled milk, for instance, is especially apt to become rancid, since the air has been expelled by boiling. Moreover, the competing lactic-acid bacteria are removed by boiling, while in fresh milk they precede in their development all other microorganisms.

The material used for butyric-acid fermentation is sugar and the chief products of it are butyric acid, carbon dioxide, and hydrogen. It may be represented by the equation

$$C_6H_{12}O_6 = CH_3 \cdot CH_2 \cdot CH_2 \cdot COOH + 2CO_2 + 2H_2.$$

Still, it practically never proceeds according to this equation, but produces a series of additional products, such as lactic and acetic acids, ethyl alcohol, and methane (CH_4). Many species of butyric-acid bacteria produce very effective enzymes capable of hydrolyzing and fermenting the most stable compounds, for example, cellulose, which usually resists the action of other microorganisms.

Bacteria of butyric fermentation are widely distributed in nature. The soil is literally crowded with them. They play an important rôle in the disintegration of the most stable plant residues. They develop at the bottom of bogs and their activity explains the production of marsh gas, consisting of methane and hydrogen, as well as phosphine, which causes spontaneous combustion of the gas with the production of "Jack o' Lanterns."

Acetic-acid fermentation differs somewhat from other kinds of fermentation. Its chief difference lies in the fact that the material used, ethyl alcohol, is not broken up, but is oxidized; hence it

occurs only in the presence of air. In this respect it shows an evident resemblance to respiration, but differs from the latter in that the products of oxidation are not carbon dioxide and water, but acetic acid. The quantity of energy liberated is consequently smaller than in complete oxidation. Acetic-acid fermentation is therefore characterized by the transformation of as large quantities of substances as are other types of fermentation.

Acetic-acid fermentation is produced by several species of bacteria, and by certain yeasts and molds. It takes place according to the following equation

$$CH_3CH_2OH + O_2 = CH_3COOH + H_2O + 117 \text{ Cal.}$$

This rather simple chemical reaction may proceed quite smoothly, even under the action of inorganic catalysts, like spongy platinum. By a process similar to that used for obtaining zymase, a special enzyme capable of oxidizing alcohol can be secured from acetic bacteria, but its action is considerably weaker than that of living cells. The bacteria causing this fermentation are considerably less sensitive than other organisms, to acetic acid or vinegar, the product of their vital activity. They can withstand as much as 6 to 10 per cent of this acid, therefore, the presence of acetic-acid bacteria in a liquid checks the development of all other microorganisms. When the supply of alcohol in the liquid is exhausted, they will proceed to a further oxidation of the previously accumulated acetic acid, ending in carbon dioxide and water. Hence in technical production of vinegar care must be taken to remove the product obtained from the place of its formation.

Acetic-acid fermentation is applied in industry for obtaining table vinegar. According to a French method of preparing vinegar, wine is poured into broad shallow bowls and inoculated with the so-called mother of vinegar, a film of vinegar bacteria. The film spreads rapidly, soon covering the whole surface of the liquid and within a few days the wine has become converted into vinegar. According to a German method, the wine or beer is allowed to flow slowly over beechwood shavings, which fill a high conical cask provided with a ventilation funnel. The shavings serve as a firm substratum on which the vinegar bacteria have settled. They oxidize the alcohol in the liquid which runs slowly over them.

All these various forms of fermentations, to which until recently was attributed a quite different chemical procedure, are regarded by Kostytchev as connected with one another and with alcoholic fermentation, showing only certain deviations from the fundamental process. In all fermentations there are formed in the first place pyruvic acid and hydrogen. In alcoholic fermentation the pyruvic acid splits up, under the influence of carboxylase, into acetaldehyde and CO_2. In the bacteria of lactic-acid fermentation, carboxylase is absent, therefore the above-mentioned cleavage does not take place. The active hydrogen combines with the carbonyl group of pyruvic acid, reducing it to lactic acid according to the equation

$$CH_3 \cdot CO \cdot COOH + 2H \rightarrow CH_3 \cdot CHOH \cdot COOH.$$

In impure cultures of lactic bacilli, carboxylase may be present but in very small quantity. As a result, a mixture of different products of fermentation is obtained.

With butyric-acid fermentation, on the contrary, the reaction leading to the reduction of the acetaldehyde is checked by the active hydrogen. The combination of 2 molecules of acetaldehyde, through aldol, into butyric acid according to the following formula is obtained:

$$2CH_3 \cdot CHO \rightarrow CH_3 \cdot CHOH \cdot CH_2 \cdot CHO$$
$$\rightarrow CH_3 \cdot CH_2 \cdot CH_2COOH.$$

The hydrogen has nothing to combine with and as a consequence is eliminated.

Acetic-acid fermentation also passes through the acetaldehyde stage. The hydrogen, however, is oxidized by atmospheric oxygen into water, while the acetaldehyde, with the addition of water, is transformed into alcohol and acetic acid:

$$2CH_3 \cdot CHO + H_2O \rightarrow CH_3 \cdot CH_2OH + CH_3 \cdot COOH.$$

Thus, Kostytchev's scheme enables us to group all forms of fermentation into one interrelated system.

83. Connection between Fermentation and Respiration. The Chemistry of Physiological Oxidation.—It has already been pointed out that higher organisms, when transferred into an oxygen-free air, display anaërobic respiration, which in its main fea-

ture is essentially the same as alcoholic fermentation caused by yeast. Therefore, the view has been established that alcoholic fermentation and respiration are closely related phenomena, the former being but a preparatory stage of the latter.

Formerly the connection between fermentation and respiration was supposed to be of the following nature: The first products formed from sugar are alcohol and carbon dioxide In an oxygen-free medium the process stops at this point, but in the presence of air the alcohol obtained is oxidized further to carbon dioxide and water. This supposition has been given up, however, since during respiration alcohol is oxidized less readily than sugar. The process is now commonly supposed to take place in the following way. Under the influence of zymase, which is always present in cells, some intermediate products of alcoholic fermentation are formed first. The subsequent fate of these products is varied. Alcohol and carbon dioxide are formed of these substances when oxygen is lacking. In the presence of oxygen they break down to carbon dioxide and water before alcohol has been formed.

Many of these chemical changes still remain obscure. Moreover, the fact that it is not known as yet which of the intermediate compounds are oxidized in presence of air creates considerable difficulties in the investigation. In the living plant, the oxidation of sugar proceeds so readily and easily, that it is impossible to determine any intermediate products. Only in certain cases, as in fleshy plants, it may be observed that respiration is accompanied by an accumulation of organic acids, but these acids are rather accessory than intermediate products.

The readiness and ease with which physiological oxidation takes place in the living cell, by no means indicates that this process is a simple one. It may be due to a harmonious cooperation of all the phases constituting it. In order to study the different phases and to destroy their connection the cells are usually killed preferably by freezing, a method that has been noted, which yields valuable results in the study of enzymatic activity.

The killing of plants for the purpose of learning their respiratory mechanism has been applied especially by Palladin and his students. He came to the conclusion, that respiration of plants has to be considered as a summation of enzymatic activities. Plants killed with sufficient rapidity and care will continue to eliminate carbon dioxide and to absorb oxygen, but these processes

diminish rapidly, since their harmony has been disturbed. Among the various substances which accumulate under these conditions, alcohol may be usually observed, whence it follows that the primary stage of respiration really represents alcoholic fermentation.

The oxidation processes following decomposition, and proceeding under participation of air, have received different interpretations by various authors. According to Palladin's original theory, slightly altered by Kostytchev, these reactions are caused by oxidizing enzymes or oxidases. The most thoroughly investigated ones are the enzymes oxidizing various aromatic compounds of the phenolic type, such as hydroquinone, tyrosin, etc. According to the idea of Chodat and Bach, the operative mechanism of oxidase consists of a peroxide, oxygenase, and a special enzyme, peroxidase. Under action of the oxidase system the peroxide decomposes into atomic oxygen, which has the power to oxidize other compounds; and a reduced fraction, which is able to unite again with molecular oxygen. The oxidase system is therefore a carrier of oxygen to substances which by themselves are incapable of being oxidized in air by molecular oxygen.

More detailed investigations on oxydases have shown, however, that their rôle in oxidation is very limited. They can oxidize only a very few substances, chiefly phenolic compounds. This seems to be a simple union with oxygen, further decomposition of the substance, with the formation of carbon dioxide, does not usually take place. This forced Palladin to introduce some alterations into his theory of respiration. He accepted the view that substances oxidized by the oxidases are merely carriers of atmospheric oxygen to the respiratory material. As the materials themselves are colorless, while the products of their oxidation are colored, Palladin named the former respiratory chromogens and the latter respiratory pigments.

Further studies have shown that the oxidation of many chromogens consists not in the union with oxygen, but in the elimination of hydrogen. Methylene blue is one of a number of this kind of pigments. It is capable of participating in oxidizing processes, though it does not contain oxygen. Its colorless compound differs from the pigment not by the absence of oxygen but by the presence of 2 atoms of hydrogen. Still it is possible to oxidize by means of methylene blue in an oxygen-free atmosphere. Thus alcohol, for instance, may be oxidized to acetic acid by using acetic

bacteria or the enzyme obtained from them. The theory of anaërobic oxidation was worked out by Wieland, who showed that it occurs at the expense of oxygen in the water molecule and that methylene blue and similar compounds are not really oxidizers but hydrogen acceptors, substances capable of uniting with the hydrogen of water and liberating thus atomic oxygen, which produces oxidation proper. The enzymes hastening these processes are not oxidases but reductases, substances binding hydrogen with the hydrogen acceptors.

Since, simultaneously with the reduction of the acceptors, the reductases induce oxidation at the expense of the oxygen or more exactly the hydroxyl group of water, it would be better to call them, as suggested by Bach, oxydo-reductases, *i.e.*, oxydo-reductive enzymes. An excellent example of such simultaneous oxidation and reduction at the expense of the elements of water, is the reaction of Cannizzaro which is produced by aldehydes in the presence of alkalies:

$$\begin{matrix} RCHO \\ \\ RCHO \end{matrix} + H_2O \longrightarrow \begin{matrix} RCH_2OH \\ \\ RCOOH \end{matrix}$$

This reaction, as we have seen, plays an important rôle in the process of alcoholic fermentation, and the mutase inducing it may be added to the group of oxydo-reductases.

On the basis of all these considerations, Palladin finally arrived at the following mechanism of processes occurring during respiration. First of all there occurs an anaërobic disintegration of both the respiratory material and water, during which the carbon contained in the respiratory material is oxidized to carbon dioxide by oxygen of the water, the hydrogen acceptors (R) binding the liberated hydrogen of the water molecule,

$$C_6H_{12}O_6 + 6H_2O + 12R = 6CO_2 + 12RH_2.$$

The second phase consists of the oxidation to water of the hydrogen bound by the acceptors. The liberation of the acceptors permits them to resume their activity,

$$12RH_2 + 6O_2 = 12H_2O + 12R.$$

By summing up both equations the usual formula for respiration is obtained.

It is evident that according to this theory all of the carbon dioxide formed during respiration is of anaërobic origin, being formed without the participation of oxygen of the air. The oxygen given off during respiration as carbon dioxide has no connection with atmospheric oxygen. Moreover, it is used exclusively for the combustion of hydrogen eliminated by the hydrogen acceptors from the respiratory material. The main enzymes of respiration are not oxidases, but reductases, serving in the transmission of hydrogen from the respiration material to the respiratory chromogens.

Palladin's ideas have found important corroboration in the experiments of Oparin. The latter has succeeded in obtaining in pure form a respiratory chromogen which is rather common in plants, namely, chlorogenic acid, a polyatomic phenol derivative. Being oxidized by atmospheric oxygen, which process takes place very readily in the presence of a special oxidase, this compound becomes a respiratory pigment, *i.e.*, a greenish-colored, energetic acceptor of hydrogen. If an amino acid is added to the solution, it will be oxidized by the oxygen of the water molecule, and the pigment will be transformed by the hydrogen to chlorogenic acid. This reaction can be accelerated considerably in the presence of the oxydo-reductase found in large quantities in the sap of sunflower seedlings. Thus, Oparin was able to reproduce artificially the respiratory process of plants: oxidation of the chromogen by means of oxidase into a pigment, and its reduction to chromogen by means of oxydo-reductase, with simultaneous oxidation of the respiratory material, in the case given, an amino acid, at the expense of the oxygen of water.

With further action of oxidase on the respiratory pigment, a deeper oxidation of the latter into a dark-brown, very stable substance takes place. This pigment cannot be reduced by hydrogen and is therefore entirely inactive; hence, it is necessary to take concentrations of oxidase and oxydo-reductase in which the oxidation processes proceeding in the solution are balanced, in order to secure a regular oxidation of the respiratory material. With an excess of oxidase, the chlorogenic acid rapidly assumes the form of a brown, inactive pigment and the reaction stops.

Similar phenomena are observed in the plant cell. Under normal conditions the oxidation and reduction processes are in perfect harmony, and the combustion of the respiratory material,

the intermediate products of alcohol fermentation, goes on smoothly. But when the normal structure of the protoplasm is disturbed, for instance by mechanical injury, through influence of narcotics, freezing, etc., then oxygen has an easier access to the cell, and the oxidation of the chromogen goes on until it results in stable, dark-colored products and the whole process is checked. This explains the cessation of respiration in dead cells, as well as their turning brown in contact with air, a phenomenon observable in cut or frozen apples, or in sap pressed from potatoes. Sometimes these stable ultimate products of oxidation of the respiratory chromogens are not brown-colored, but of more brilliant colors. Thus, for instance, the fruiting bodies of many fungi turn blue, green, or red at the broken surfaces.

Palladin's theory has not been verified conclusively and will no doubt undergo revision in the course of further investigations. In recent years, however, new evidence in favor of this theory has been supplied through the work of chemists, as well as of plant physiologists. This theory has been applied to the physiology of animals, because essentially respiration is the same phenomenon in the whole organic world. Then, too, it is in harmony with the modern ideas as to the rôle of water in all kinds of oxidation processes.

The nature of respiration has been considered by Warburg from quite a different point of view. On the basis of a number of observations, he attempts to prove that respiration is connected with the presence of a permanent colloidal structure. He considers it to be essentially an adsorption process, taking place on the surface of the colloidal particles of protoplasm. Warburg succeeded in constructing an artificial model of respiration. He used blood charcoal as an adsorbing body with a large surface, and as respiration material a solution of cystine and other amino acids. In the presence of oxygen, such a model showed a resemblance to the respiration process with absorption of oxygen and formation of CO_2, H_2O, NH_3, and SO_3.

Iron was found to be an exceedingly important agent in the working of this respiration model. A prominent rôle in physiological oxidation has been ascribed to iron for some time, but it could not be proved by precise determination. Warburg's experiments have shown that respiration in his model is one-third to one-half as fast, when, instead of using blood charcoal, which always

contains a small amount of iron, a special charcoal containing very little iron is used as a substitute; but by addition of traces of iron the oxidizing power of such a charcoal is greatly increased. Warburg's experiments on the effect of narcotics, such as ether, chloroform, etc., have been of great interest. These substances depress considerably the rate of respiration in living cells. Their influence on the charcoal model is the same. A complete parallelism has been observed between the narcotic action and the capacity of adsorption, which, in its turn, is proportional to the decrease of surface tension. The only exception to this rule is shown by hydrocyanic acid, which even in very weak concentrations markedly depresses respiration, though it is scarcely adsorbed at all. This exception, however, is easily explained by the fact that HCN unites readily with iron, forming a complex compound and thereby removing it from the sphere of the oxidizing reaction.

Warburg's conception of respiration, as a process occurring on the surface of the colloidal particles of protoplasm rich in iron, appears very attractive. It explains satisfactorily many peculiarities of the respiratory process. The participation of enzymes in respiration by no means is excluded, since they too represent colloidal catalyzers having an enormous surface. Further investigations may point to the best way of correlating the absorption theory of Warburg with the enzyme theories of respiration. It is to be hoped that in the next few years considerable progress will be made in determining the internal chemistry and mechanism of this fundamentally vital process.

84. Influence of External and Internal Factors on Respiration and Fermentation.—Representing as it does a very complex and highly harmonious oxidation process, respiration is very little dependent on external influences. It is regulated chiefly by internal conditions. Of the latter, the most important is the presence of respiratory material, which, as has been seen, consists chiefly of sugar. The rate of respiration is, therefore, rather closely connected with the supply of carbohydrates. It decreases considerably under conditions of starvation. This explains the relation of respiration to exposure to light, established by Borodin in one of his first experiments. When shoots with leaves are kept in darkness, their respiration rate gradually decreases as the carbohydrates become exhausted. Upon exposure to light the supply of carbohydrates is restored and respiration rapidly increases.

Intense respiration requires also the presence of a sufficient quantity of respiratory enzymes in the protoplasm. The rate of respiration is, therefore, highest in younger parts of a plant. In the older portions, especially the storage organs, respiration is very low, in spite of an abundance or respiratory material. The amount of respiratory enzymes produced by protoplasm may vary as a result of several causes. Thus, for instance, when potato tubers and other storage organs begin to sprout, they will respire with greater energy than in a dormant state. Various kinds of irritations, as cutting and exposure to poisonous substances and sharp changes in temperature, likewise will cause a considerable increase of the respiratory rate.

Of the external factors directly influencing respiration the most important is the effect of temperature. Temperature is the general regulator of the rate of chemical processes. Its influence, (see Art. 14), may be represented by Van't Hoff's law, according to which the rate of a reaction almost doubles with an increase of 10° C. in temperature. The lowest limit at which respiration may still be observed, at least in hibernating plants, for instance, buds of deciduous trees and needles of conifer, is −20 to −25° C. Differing from assimilation and many other vital processes in which, after a certain optimal point is reached, a further rise in temperature leads to a decrease of the process, the intensity of respiration increases continually until the killing point is reached (about 45 to 50° C.). This uninterrupted rise of respiration proceeding along with a decrease in the rate of assimilation with an optimum at 30° C. leads to a debilitation of the organism at high temperatures. In cultivated plants, therefore, it is frequently expedient to keep the temperature at a comparatively low level, especially when the light is not sufficiently intense and prolonged, as in greenhouses during winter. Otherwise, respiration will exceed too greatly the process of assimilation, which is very feeble under the conditions of insufficient light; the plant will rapidly spend the reserves accumulated in summer and may finally perish from exhaustion.

The relation between the rate of assimilation and that of respiration plays an important rôle in the life of plants, since the difference between these two processes determines the amount of accumulated organic substances. Under normal conditions of light in open exposures, the rate of assimilation is several times higher than that of respiration and there is a general positive

balance. In deep shade, on the contrary, respiration usually exceeds assimilation and, instead of accumulating organic substance, the plant is consuming them. Finally, there may be obtained an intensity of light under which respiration will just balance assimilation. In this case, the plant will produce no change in the composition of the atmosphere, since all of the carbon dioxide eliminated during respiration will be immediately used up in assimilation, and all the oxygen eliminated during assimilation will be consumed in respiration. This intensity of light is called the "compensation point." Its position determines to a certain degree the light requirement of a plant. The rate of respiration of shade plants is in general considerably lower than in light-loving plants. Still, in spite of a feeble intensity of light, their assimilation proceeds at a comparatively higher rate.

Light by itself has no perceptible influence on the respiration of plants, without chlorophyll. When the intensity of light is very great, however, there may be an effect as a result of the increasing influence of a high temperature on respiration and the destructive action of light on protoplasm.

Within a fairly wide range, the amount of oxygen in the air has no perceptible influence on the intensity of respiration. This is in complete accord with the views on the chemistry of respiration, according to which the first phase of respiration proceeds without the participation of free oxygen. Only when the amount of oxygen has decreased to as low a point as 1 to 2 per cent, the respiration coefficient begins to increase perceptibly. Alcohol then accumulates in the tissues of a plant, which is a sign that the oxidation begins to lag behind the processes of anaërobic disintegration. Hence, no reduction in respiration is usually observed, even in massive plant organs in the presence of sufficiently developed intercellular spaces. In the case of seeds maturing within a fruit or during germination of seeds having a very compact coat, aëration may be insufficient, resulting in retardation of growth. Even here, it is not the deficiency in oxygen that is of importance, but the excessive accumulation of carbon dioxide which acts like a narcotic. An excess of oxygen, for instance, with a considerable increase of air pressure, as in the case of placing the plant into an atmosphere of pure oxygen, may also prove detrimental, by inducing excessive oxidation and the inactivation of the respiratory chromogens.

The influence of external conditions on alcoholic fermentation is, on the whole, the same as on respiration. As the temperature rises, fermentation rapidly increases, reaching its maximum at 50° C., when yeast begins to die. Light somewhat retards fermentation, as it curtails the vital activity of many lower organisms. The greatest interest is attached to the study of the influence exerted on fermentation by carbon dioxide and oxygen. Being one of the products of fermentation and accumulating in large quantities in the fermenting liquid, carbon dioxide has no evident influence on fermentation. Yeast is as little sensitive to it as to the accumulation of alcohol.

The presence or absence of oxygen is of little importance in the process of fermentation. Yeast ferments sugar in an anaërobic medium, as well as in the air. With oxygen, however, yeast undergoes oxygen respiration, producing as has been seen, a much larger amount of useful energy. For this reason, yeast ferments a somewhat smaller amount of sugar in the presence of air than under anaërobic conditions. This economizing influence of oxygen is different with the various yeast races. It is less marked in beer yeast and more marked in wine yeast that lives in the wild state on the surface of grapes, *i.e.*, under the conditions of an abundant air supply.

One must distinguish the direct influence on fermentation, as on enzymatic process, from the influence of external factors on the reproduction of yeast. In the industries, of course, the greatest importance is placed on the reproduction of yeast in the fermenting liquid, since, other conditions being equal, the rate of fermentation of a liquid is dependent upon the number of cells producing it. Here the oxygen plays a very important rôle, since in its presence reproduction proceeds much more rapidly than without it. For the same reasons it is also advisable to remove the carbon dioxide which usually accumulates in the liquid. Hence, in the beer-brewing industry fermentation proceeds in flat vats, or else air is blown through the fermenting liquid. Moreover, the optimum temperature for the reproduction of yeast is 25 to 30° C., not 50° C.

Part IV

GROWTH, MOVEMENT, AND REPRODUCTION

CHAPTER X

GROWTH

85. General Laws of Growth and Their Mathematical Formulation.—In considering the growth of plants it is customary, first of all, to emphasize the increase in size of growing organs, growth in height often being distinguished from growth in thickness. Increase in size, however, is not always connected with an increase in the amount of organic substances. It has been emphasized that seeds growing in darkness may actually lose in total dry weight.

A general increase in size of plants is, therefore, connected not so much with the accumulation, as with a redistribution of organic substances. During growth, the reserve materials are transformed into soluble forms which go into the building of the plastic substances constituting living protoplasm. Accretion in the amount of protoplasm results in an increase of the number of cells and the general dimensions of a plant. It must be noted, though, that not every increase in size should be termed growth. The swelling of seeds, for instance, is not growth. By growth must be understood only such an increase in size that is permanently acquired through internal processes.

An exact study of the phenomenon of growth requires, in the first place, a precise measurement of it. Several methods are available for this purpose. The simplest of them consists in measuring the length of the growing organs or of whole plants at definite time intervals by means of a properly subdivided ruler. As the growth of plants on the whole is rather slow, this comparatively crude method may be used only when measurements are taken not oftener than every 24 hr. When it is desired to follow the growth of a plant at shorter intervals of time, a horizontal microscope will be found convenient. It is focused upon the end of the growing organ, as for instance, the tip of a blade of grass or the end of a root, and then by means of an ocular micrometer the

distance covered by the extremity of the plant is determined (Fig. 105). Knowing the magnification of the microscope, it is easy to calculate the growth increase. It is possible to use a self-recording apparatus, the so-called auxograph in which, by means of pulleys and wheels, changes in the position of the tip of a plant are recorded on an enlarged scale on the surface of a revolving smoked paper

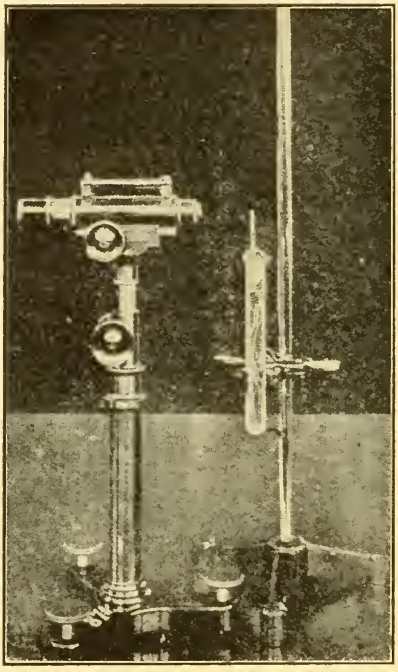


FIG. 105.—Measurement of growth by means of a horizontal microscope (*after Molisch*).

cylinder (Fig. 106). Auxographs usually give an enlargement of twenty to forty times. From such records one can read alterations in growth, occurring in the course of half an hour or an hour. Recently, however, the Indian scientist Bose has constructed an apparatus, the crescograph, which gives enlargements of 1,000 and even 10,000 times. This apparatus allows one to record the progress of growth not only by minutes but even by seconds.

Often it is important to follow the growth of certain organs instead of that of the whole plant. In such cases, Sachs' method of

marking the growing organs by means of India ink applied with a fine brush is used. These marks are made at definite distances

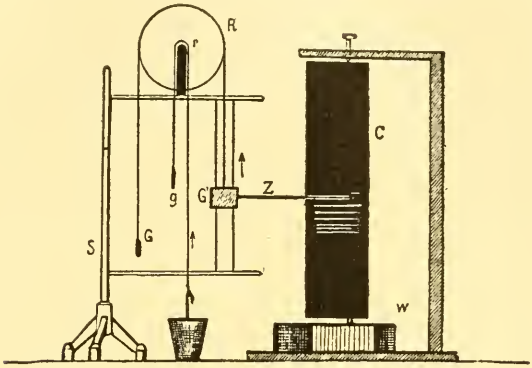


FIG. 106.—Wiesner's auxograph (*after Molisch*).

from one another and the increase in the distance is measured at certain time intervals. This enables one to obtain a precise

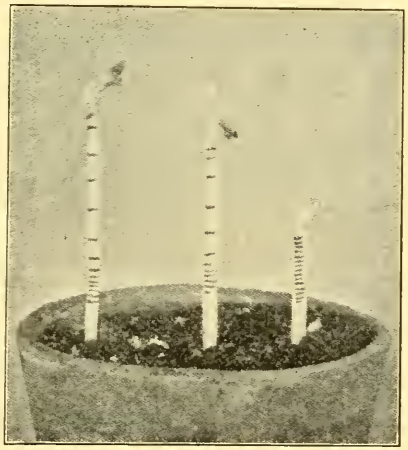


FIG. 107.—Two etiolated seedlings of the horse bean with markings made 24 hr. before photographing. On the right, a seedling with markings just made (*after Nathansohn*).

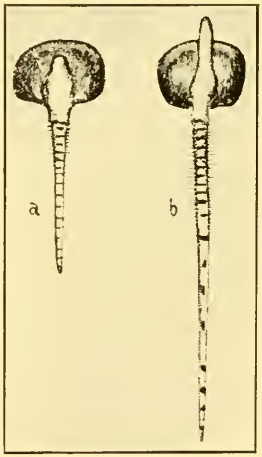


FIG. 108. — Maize sprouts with markings made on roots: *a*, before the experiment; *b*, 24 hr. later (*after Molisch*).

measurement of the change in length of each portion during the experiment (Figs. 107 and 108) and to ascertain the dimensions of

the entire growing zone, since only in this region the distance between the marks will increase. On the basis of growth increments of separate regions, as well as whole organs, Sachs established a definite law of "the grand period of growth." According to this law, every organ and each definite region of a developing organ at first grows slowly, then accelerates, reaches its maximum speed, and finally slows down. The following figures show daily growth increments of a small section in the growing roots of the horse bean, which at the beginning of the experiment was 1 mm. in length.

Days	0	1	2	3	4	5	6	7	8
Growth increase, millimeters...	....	1.8	3.7	17.5	16.5	17.0	14.5	7.0	0.0
Total length, millimeters.....	1	2.8	6.5	24.0	40.5	57.5	72.0	80.0	80.0

In the bottom row are given the figures of the whole length of the region at the moment of its measurement. If the results of these measurements are represented graphically, two distinct curves are obtained (Fig. 109). The curve of increments has an almost symmetrical aspect with a rather prolonged maximum (dotted lines), while the curve of changes in the total length of the section is of the letter S type. It rises, at first, slowly, then more rapidly, and finally approaches the horizontal direction.

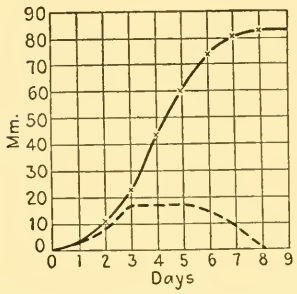


FIG. 109. — Growth curves of roots of *Vicia faba* (*after Rippel*).

In this growth curve one can distinguish two sections: from the beginning to the maximum, or the most rapid rise, and from the maximum to the cessation of growth. The first part may be termed the initial phase, since here we have the manifestation of growth in its purest aspect. The second part may be considered as the development of retardation, which shows itself at first weakly but later with increasing force, until growth ceases. For the initial phase, Blackman applies the formula of compound interest, since, in growth we are dealing with an increase in the number of cells following a geometrical progression, in which one cell forms 2, 2

producing 4, then 8, 16, 32, 64, etc. Designating by the letter A the final size of the section, and by a the initial one; by t, the time interval; and by r, the rate of percentage increase, the formula $A = aert$ is obtained, where e is the base of natural logarithms. Changing this formula to a logarithmic form and converting the natural logarithms into decimal ones, we obtain.

$$2.3026 \log \frac{A}{a} = rt.$$

whence it is easy to compute the percentage of growth per day, the initial and final size, and the number of days being known.

Blackman's formula is applicable only in a limited way to the initial phase of the curve, for the exact moment of retardation is very difficult to determine. Since growth is based on the chemical transformation of assimilated or reserve substances into living molecules of protoplasm and the latter also begin to participate as soon as they are formed, in the process of growth, Robertson has made an attempt to apply to growth the formula of the monomolecular autocatalytic reaction which should embrace the entire process from beginning to end. If one considers the gradual retardation of the reaction as being due to the accumulation of its end products, then the formula may be represented by $\frac{dx}{dt} = K \cdot (A - x)$, where K is an empirical constant, x the size reached in t days, or other time units, from the beginning of growth, and A the final size of the growing organ. By integrating and other transformations Robertson deduces from this formula another, which is more convenient for calculation:

$$\log \frac{A - x}{x} = k(t - t_1),$$

in which t_1 represents the time necessary for the growing organ to reach one-half its final size. For many calculations it is convenient to take relative values instead of absolute ones, and to assume the final value of A to be 100.

Besides Blackman's and Robertson's formulae, others have been proposed. But all of them fail to express adequately the progress of growth, since, in addition to internal conditions, the increments per time unit depend also on environmental factors, such as tem-

perature, light, supply of nutrient substances, etc. Consequently, these formulae represent the process of growth only approximately, Robertson's formula having the advantage that it attempts to cover the whole period.[1]

The causes which retard and check growth are not fully understood at present. Here one must consider the general problem of senescence of an organism, which is still unsolved.[2]

86. Growth of Individual Cells and Its Mechanism. The Three Stages of Growth: Embryonic Development, Expansion, and Internal Differentiation.— Fundamentally, the growth of plants and their organs consists of the multiplication and growth of the cells composing them. Therefore, in order to understand the laws of growth, the alterations occurring during growth in the cells must be studied. Not all the cells of a plant, however, participate in its growth. The greater part of the plant consists of mature cells. Only in certain regions are there found meristematic tissues consisting of growing cells, or tissues capable of growth and multiplication. These are the so-called growing points, forming the tips of stems and roots.

A detailed description of the structure of embryonic regions and meristematic tissues in general may be found in textbooks of plant anatomy. Here it will be indicated only that their cells are comparatively small, have thin walls, and are completely filled with protoplasm containing a large centrally located nucleus. Growth of meristematic tissues proceeds in the following manner: The amount of protoplasm in the cells gradually increases, resulting in an increase in size of the cells themselves. Thereupon, division of the nucleus and of the protoplasm takes place and the newly formed cells are separated from each other by new walls. The accumulation of living substance begins anew, followed by a new division of cells. Consequently, in the meristematic region the fundamental process is an increase in the number of cells and in the general mass of living matter.

In organs with uninterrupted growth, such as roots or young stems, the total mass of meristematic tissue remains constant, in spite of the continuous division of its cells. This is due to the fact

[1] Of only certain kinds of plants, primarily those with a discontinuous type of growth, such as the sunflower, wheat, etc.

[2] Correlation between vegetative and reproductive functions often determines the character and rate of growth.

that in the lower part of the growing region the meristematic cells pass into the second stage of growth. This is termed "elongation." This stage is characterized by the appearance of vacuoles in the protoplasm, which become filled with a watery content, the cell sap. These vacuoles enlarge rapidly, and finally nothing is left of the protoplasm but a thin lining appressed to the walls. The whole cavity of the cell constitutes now a large

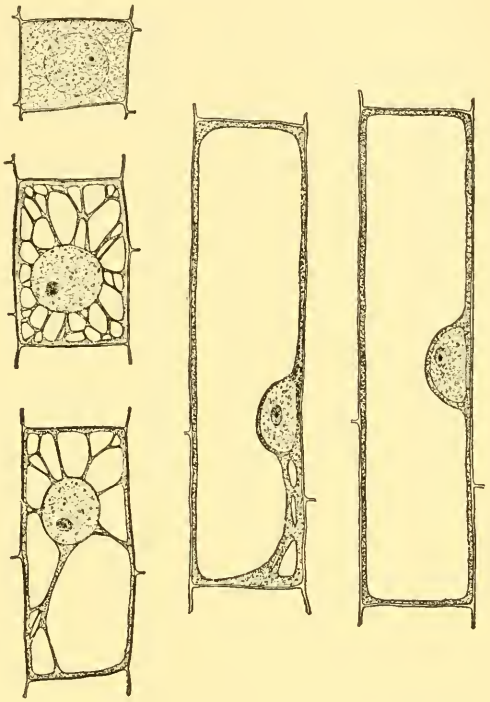


FIG. 110.—Successive stages in the growth of root cells (*after Brown*).

central vacuole. The volume of the cell naturally has increased, the walls having grown considerably, but the amount of protoplasm apparently remains unaltered (Fig. 110).

The enlargement of the cell during the stage of elongation results from different causes than those operating during meristematic growth. There we had to deal with an increase in the amount of protoplasm; and here with an increase in the quantity

of cell sap, consisting mainly of water. The question naturally arises as to what causes water to accumulate in the cell and to increase the cell volume. The process of cell elongation, which sometimes had been identified with the mechanism of cell growth was considered for a long time to be as follows: It was supposed that there appear in the cell osmotically active substances like sugars or organic acids. These substances then begin forcefully to attract water, resulting in an increased turgor of the cell. The still thin and yielding cell wall will begin to stretch under the influence of turgor pressure, similar to a rubber balloon when air is pumped into it, and the volume of the cell increases. At the same time, there occurs a dilution of the osmotically active substances, resulting in a decreased osmotic pressure within the cell and a gradual lowering in turgor. The stage of elongation then terminates.

This idea, appealing in its simplicity and permitting one to compare the growth of living cells with the so-called artificial ones prepared from precipitation membranes, for a long time enjoyed great popularity. Soon after its rise, however, facts began to accumulate, which contradicted it. It was found that osmotic pressure does not decrease in cells as they grow, but remains fairly constant. Moreover, Ursprung's recent researches have shown that in the period of elongation cells do not have a greater but, on the contrary, a lower turgor and at the same time a considerably increased suction tension. With the osmotic pressure unchanged, an increase in suction force can take place only when there is a diminished pressure of the cell membrane (Art. 42). The volume of the cell increasing, this decrease can occur only by a rapid growth of the membrane itself.

Ursprung's investigations enable us to construct the following mechanism of the growth of cells in the stage of elongation. The increase in amount of protoplasm, characteristic of the embryonic stage, is replaced by an augmented production of cellulose and other substances of the cell wall, thus causing the membrane to grow rapidly. Its pressure on the cell contents now diminishes, and as a result there arises a considerable suction tension, causing water to enter the cell. This suction is so great, that in case of shortage young growing parts may draw water from old, already mature cells. This explains the fact that young shoots of the potato and other tuber and root crop plants can absorb water from the storage

tissues on which they develop, although the absolute degree of their osmotic pressure is not great.

The growth of cells in the zone of elongation is closely related to the activity of the region of embryonic tissue which lies above it. If the growing point of a stem is cut, then the elongation of the lower zone immediately decreases and is accelerated only after the process of regeneration starts at the place where the cut was made. This slowing down of growth is especially conspicuous on flowering stems when the flower buds are removed, and also on coleoptiles of cereal plants that are just breaking through the soil. If the severed growing point is attached again by means of a drop of water or gelatin, then growth is notably increased. Recent investigations by Went (1927) show that the growing point of a coleoptile manufactures a special substance, called by him a "growth-promoting substance," which descends in the growing organ and causes stretching of its cells. When the severed growing point is placed with its cut surface on a thin layer of agar gelatin and left for about 2 hr., the layer of agar absorbs this growth-promoting substance. If the growing point is removed from the gelatin and a piece of the latter is placed on the cut surface of the coleoptile, then growth immediately increases. Wendt has even determined the rate of diffusion of this growth-promoting substance in agar gelatin and also its approximate molecular weight, which was in the neighborhood of 350, or approximately equal to the molecular weight of sucrose. Further than this the chemical nature of the substance is not known.

This growth-promoting substance can be formed not only in growing tips but also in other parts of vegetative organs. According to Cholodny, it is formed in the phloem of the hypocotyl of the lupine and sunflower. If, by means of a special drill, the central cylinder containing the phloem elements of a section of the hypocotyl is removed, the rate of growth of the section is noticeably decreased. If now a tip cut from the growing point of the coleoptile of corn is placed into this hollow stem, the growth of the hypocotyl will increase considerably, since it is under the influence of the growth-promoting substance secreted by the tip of the corn coleoptile.

From these experiments one may conclude that the growth-promoting substance is not specific, as the secretion of the tip of coleoptile of corn also increases the rate of growth of a lupine or sun-

flower plant. The same result may be obtained by using tips of dandelions or flowering stems of poppies on coleoptiles of oats.

The growth-promoting substance moves along the stem in a definite direction, *i.e.*, from the tip to the base, independent of how the organ is oriented in space. According to Went, the mechanism of its action consists in softening of the cell walls, which facilitates their stretching. It is possible that this substance is an enzyme. The substance is used up in growth, the lower zone containing less than the upper one. Went believes that the presence of this substance determines the period of maximum growth. It is interesting to note that the tip of a root secretes a substance which slows down rather than increases the growth of the upper zone of this organ. It appears, however, that the difference is not due to the properties of the substance secreted but to the peculiarities of the cells of roots in the zone of stretching. Experiments of Cholodny show that a root tip increases the growth of decapitated coleoptiles; and, on the contrary, the tips of coleoptiles hinder the growth of decapitated roots. From this it follows that the growth-promoting substance, or as Cholodny calls it, the growth hormone, is evidently the same in all growing organs, but the various organs react differently to its presence.

At the present time, many experiments are being tried with various substances to determine their ability to increase the growth of decapitated vegetative organs. It may be noted that even saliva causes an appreciable increase in the growth of oat coleoptiles and stems of other plants.

Elongation is followed by the third and last period of growth, the stage of internal differentiation. The thin and stretched wall now grows in thickness. The cell has ceased to increase in volume and assumes its final form and size. The homogeneous zone of growth gives rise to the various tissues of the mature organ, such as the conducting and mechanical elements, etc. The internal factors, which control differentiation, frequently accompanied by the formation of special thickening in the cell walls, the loss of the cell contents, etc., remain unknown at present.

87. Periodicity of Growth. The Rest Period. Methods of Forcing Plants.—Frequently all three stages of growth succeed one another uninterruptedly. This results in even and continuous growth. Such, for instance, is the growth of roots, in the longitudinal section of which there may be found cells in all three stages

of development. At the tip, the cells are in the state of embryonic growth, 7 to 8 mm. back of it they are in the condition of elongation, and still farther back they are differentiating (Fig. 111). The zone of growth in roots is usually exceedingly short, about 8 to 10 mm. This apparently is conditioned by the necessity of the root of making its way through the solid soil. This short region of growth of a root is often compared to a short nail driven into a hard board, where a long nail would bend. In stems as well as in aerial roots

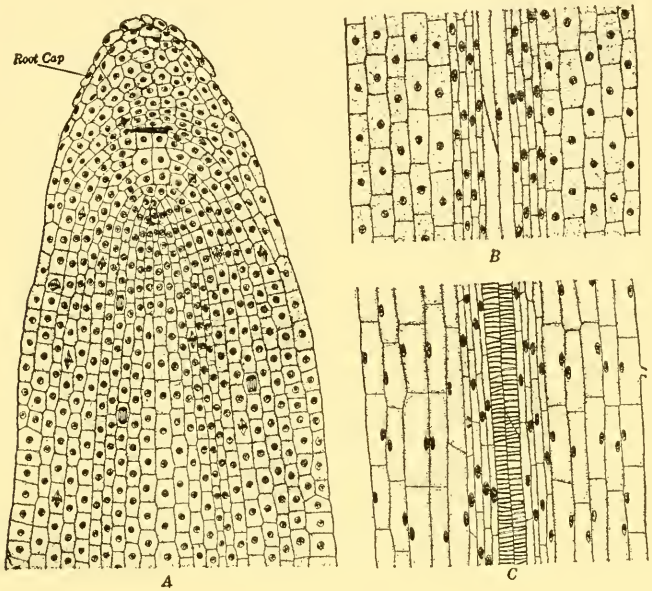


FIG. 111.—Vertical section of a growing root. *A*, root tip in meristematic stage; *B*, a higher portion in the stage of elongation; *C*, a still higher portion in the stage of differentiation (*after Smith et al.*).

peculiar to certain plants, especially of the aroid family, the zone of growth is considerably longer, often as much as 10 cm.

These stages of growth, however, do not always follow each other directly. Often they are separated by a considerable interval of time, most frequently between the stages of meristematic growth and of elongation. This condition is especially evident in the cereals. During the first weeks of development, their stems remain short, forming the so-called tillering node. Only the leaves seem to be growing then. But when the internodes and

the inflorescence have completed their embryonic development, then meristematic growth commences and a rapid elongation of the internodes sets in. At this period the inflorescence comes out of the tubelike leaf sheaths which till then have tightly enclosed it (Fig. 112).

In some cereals, these two stages are separated by the winter period, when growth completely ceases and the plant enters into a state of rest. This condition may be of various intensity and duration. It may involve the whole plant or only parts of it. The best investigations have been made on the dormancy of buds and branches of trees. In early summer, soon after the shoots and young leaves have expanded, new buds arise in their axils. They gradually increase in size during the summer months, passing through the stage of meristematic growth. Towards the end of the growing season, all parts composing the next year's shoot may be distinguished already in the buds. Frequently, even the whole flower cluster is present. But in this period meristematic growth is not followed by elongation. During the fall and winter these buds are in a state of rest.

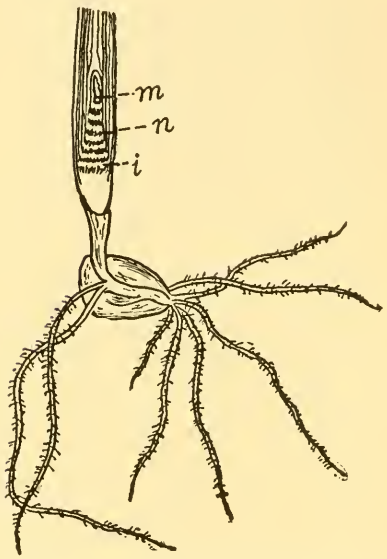


FIG. 112.—Terminal bud of a young maize plant.

One must not imagine that in this state there is a complete cessation of all vital processes. Respiration continues in all resting organs. The transformation of reserve substances also takes place. In the twigs and buds of trees, for instance, starch usually disappears during the winter and in its place sugar and sometimes fat accumulate. Toward spring reverse metabolic changes may be observed. In general, all vital activities manifest themselves during dormancy with the exception of growth, which does not take place, though the external conditions may be quite

favorable for it. One cannot make the potato tuber sprout in September and October even under favorable temperature and moisture conditions, while in spring it will develop in a cold dry room.

Growth of dormant organs, therefore, is not controlled by external inhibiting factors. It is not known what these factors are, but there are reasons to suppose that the presence of some substances opposing growth exist, the nature of which is not yet understood. Still, there are several means of removing these inhibitors and of inducing resting organs to resume growth. Some of these methods have found wide application in horticulture for the forcing

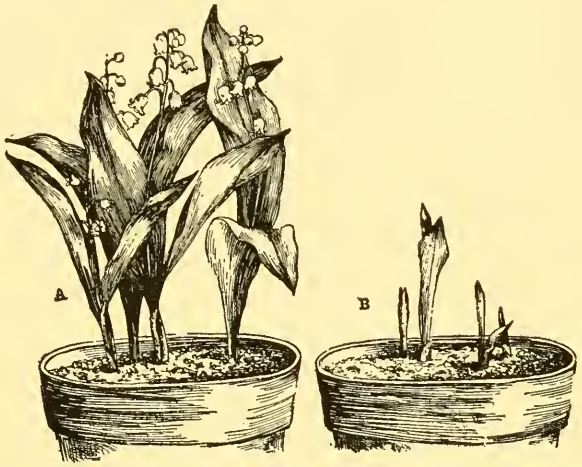


FIG. 113.—Lily-of-the-valley treated with ether, *A*, and control, *B* (*after Duggar*).

of early blossoming of perennial plants. The most important of these [1] is a treatment with ether, discovered by Johannson, and the exposure to warm baths suggested by Molisch.

Plants intended for treatment with ether, such as shrubs of lilac, rhizomes of lily-of-the-valley, etc., are placed in air-tight wooden boxes with closely fitting doors into which at the same time is placed a small cup of ordinary ether. The concentration of ether is usually 0.5 cc. per liter of air. The plants are allowed to stand in this vapor for 24 or 48 hr. after which they are transferred to a warm greenhouse. Soon the treated plants will open their buds

[1] In America, the more effective treatments by ethylene, ethylene chlorhydrin, and similar compounds are used.

and begin to bloom, while the controls, though receiving an identical treatment except being subjected to the action of ether, will remain in full dormancy (Fig. 113).

Exposure to a warm bath is still more simple. The aerial parts of the plants are immersed in water at 30 to 35° C. and 9 to 12 hr. (Fig. 114) after which the plants are transferred to a place favorable for growth. It is interesting to note that the effect of warm baths and of ether are purely local. Accelerated development takes place only in buds and branches which have been exposed directly to the stimulants. When a lilac bush is placed in such position that only part of its branches will be in warm water, the others remaining outside, then in a few days a sharp difference between the development of these branches will be observed (Fig. 115).

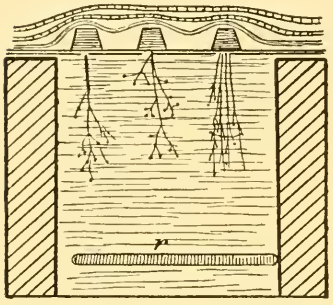


FIG. 114.—A tank for warm baths, heated from below by a radiator, *r*, and covered with felt, *s* (*after Molisch*).

Besides the treatments with ether and warm baths, several other methods to induce plants to break their rest period have been recently discovered. Plants may be forced by various volatile substances, such as prussic acid, thymol, camphor, acetone, ethylene, illuminating gas, tobacco, and other fumes. Cutting of the buds, injection of water or salt solutions, exposure to various rays and radium emanation, and many other external influences will hasten the breaking of buds. So, too, a considerable acceleration in development will be observed after freezing. Plants which have remained during the winter in a warm room will start to grow later than those subjected to the action of frost (Fig. 116).

The mechanism of the effects of these stimulants is still unknown. Some authors are of the opinion that they increase the permeability of protoplasm and thereby induce growth. Others hold that they increase the oxidation processes in the cells and thus stimulate its vital activities. By studying the effects of ether treatment at different times in the fall and winter, Johannsen came to the conclusion that its influence is not the same at different periods. This he considers an indication that the state of rest likewise varies. He distinguishes three conditions of rest: a pre-

liminary one, a stage of deep rest and a condition of forced rest, which gradually and imperceptibly merge one into another. During the preliminary stage, through which most buds pass in summer, they may be readily returned to active life. This may be accomplished simply by removing the leaves. In some trees, as oaks and elms, the buds frequently open in summer, even without special treatment, and produce secondary growth (St. John's shoots). In the fall, after the leaves have normally dropped, dormancy is

FIG. 115.—Shoot of lilac. Right branch subjected to a warm bath, left one remained untreated (*after Duggar*).

in its deepest stage. At this time forcing requires particularly strong stimulants. Towards late winter and early spring, buds gradually leave the state of rest. When placed under conditions favorable for growth, they will begin to unfold without any particular treatment. Since the absence of growth during this period is not due to internal inhibition, the usual stimulants will not hasten the unfolding of buds.

88. Seed Germination.—The mature seed is also in a state of rest. Its germination means the change to active life. Like in buds, this rest may be light, or deep. Accordingly, many seeds

germinate as soon as they are placed under favorable conditions of moisture and temperature, while others will not germinate under the same conditions for many weeks, months, or even years.

Various causes are responsible for this delay in germination, just as there are manifold methods by which germination may be hastened. Very often there is exhibited the same phenomenon of deep rest or dormancy which has just been discussed in considering buds. In such cases, seeds acquire the capacity to germinate rapidly only after having been kept for a sufficiently long time, or else they require some external stimulant.

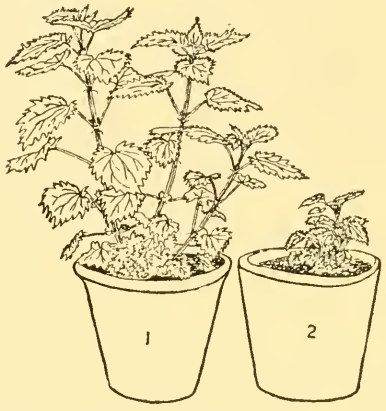


FIG. 116.—Nettle kept during the winter (1) under snow, (2) in a warm greenhouse.

An increased power of germination is often observed in winter wheat and other cereals which sometimes appear incapable of germinating immediately after harvesting. A few weeks or months later, however, they are able to germinate quickly and uniformly. The phenomenon has been termed "after-ripening." This characteristic is exhibited by various varieties in different degrees. In regions with a very wet autumn, varieties of wheat with a long rest period may be of advantage in that their grains will not germinate in the sheaves in rainy weather.

Because of the necessity of seeds to pass through the rest period, there have been developed methods of stratification, so widely used in horticulture. The mature seeds of the apple, pear, and stone fruits are usually planted in layers with moist sand and transferred to a cool place, the best temperature being 5° C., for instance to a cellar, or they may be left under snow. Toward spring the rest period will be completed, and the seeds will germinate. Instead of burying in moist sand, the seeds of apples, for instance, may be kept simply by leaving them in the flesh of the fruit. In dry seeds of these plants the process of after ripening does not take place.

The rest period of mature seeds may be so deep that they will

refuse to germinate without exposure to special stimulants even after prolonged storage. Thus, for instance, the seeds of the gooseberry, of *Lunaria*, of the Siberian *Impatiens* and other plants require more than a simple stratification. They must be frozen at −5° C. or even −10° C., to induce germination. Light is a stimulant to many seeds which fail to germinate, or which germinate but poorly in darkness. To this group belong seeds of *Poa*, tobacco, carrot, *Oenothera*, and many other plants. On the contrary, light retards germination of seeds of some plants, like those of the thorn apple, *Amaranthus*, etc. Hence they must always be germinated in darkness. What the stimulating effect of light on the germination of seeds consists in, is as yet quite obscure. The action of light may be substituted by fluctuating temperature, within the range of a few degrees, or by various chemicals, among which the most important are the acids and alkalies. Seeds of bog plants, like *Alisma*, *Sagittaria*, and others, will germinate only in an acid medium. Those of certain parasites, as the broom rape (*Orobanche*), also require an acid medium. Others are stimulated by solutions of neutral salts or substances like bromine, iodine, and certain antiseptics.

The increasingly popular treatment of seeds with various solutions, such as formalin, copper sulphate, and various patented preparations, in order to control smut and other fungus diseases, sometimes results in stimulated germination, but more often it leads to retardation.

A prolonged delay of germination does not always depend on the state of dormancy in which the embryo of the seed happens to be. Very often it is due to the properties of the seed coat. Many plants produce so-called hard seeds, which fail to germinate, because their coats are impermeable to water. Such seeds very often occur in clover, alfalfa, lupine, and other representatives of *Leguminosae*. If a certain amount of seeds of such plants is placed in water, part of them will rapidly swell and germinate, others will remain for a long time in water without swelling. Only gradually, after some weeks or months, a few seeds will absorb water and germinate, apparently, as a result of a lesion in the outer layers of the seed coat. Thus, Molisch observed in one of his prolonged experiments with the seeds of the Japanese *Gleditsia*, that 4 seeds out of 50 placed in water had swollen on the next day; 11 more within the first two months, 21 additional during the first year, 6 in

the second year, 6 more during the third year, 3 in the fourth and fifth years, while 3 seeds did not swell after having been in water for more than 5 years.

The impermeability of the coats of hard seeds to water depends on very close union and drying of the cells forming the outer layers of the seed coat. When these outer layers are injured by scratching with a knife or by filing or simply rubbing them with sand, swelling and germination will result. The corroding action of strong sulphuric acid also may be used for this purpose.

Not always, however, is the delayed germination of viable seeds due to the impermeability of their coats to water. Very often an impermeability of the seed coat to gases is the real cause, as a consequence of which the carbon dioxide produced during respiration is not eliminated, but accumulates within the seed and inhibits the active growth of the embryo. Such seeds may be kept for years without germinating, though they swell readily. The seeds of many of the common weeds, like plantain, black mustard, shepherd's purse, wild oats, chickweed, etc., are of this type. In order to make them germinate readily, it is not sufficient merely to scratch their coat. It must be split, to permit the escape of carbon dioxide and admit oxygen. In a similar condition may be seeds which happen to be covered by a thick layer of earth. They may remain alive in the ground for many years without germination, but will germinate rapidly as soon as they have reached the top of the soil. Thus, for instance, in samples of soil taken from a depth of about 20 cm. in a forest growing on ground that 30 to 40 years ago had been occupied by a field or a meadow, seedlings of weeds or meadow plants could be obtained. Evidently the seeds had retained their power of germination throughout this long period.

Such long preservation in moist ground without decaying is possible only because the seed coat eliminates on its surface some antiseptic substances inhibiting the development of bacteria and molds. It is interesting to note, that in dead seeds this elimination soon ceases and they rapidly become the prey of microorganisms which produce decay.

As the seed matures the impermeability of its coat gradually increases and desiccation often makes it still more impermeable. This explains the commonly observed phenomenon that in a fresh supply of seeds the percentage of hard seeds is somewhat lower than

in those that have been stored for a long time. It also accounts for the fact that unripe seeds of many weeds germinate better and more rapidly than ripe ones. The ready germination of immature seeds which normally pass through a period of rest, as, for instance, the seeds of winter wheat, is most likely due to the fact that they are in a condition of preliminary rest, which is more readily disturbed than a deep rest.

Having emerged from the state of rest, the axillary organs, the roots and plumule of the embryo, begin to grow, and the reserve substances deposited in the endosperm and cotyledons are consumed in the processes of respiration and growth, which, as has been seen, are closely connected.

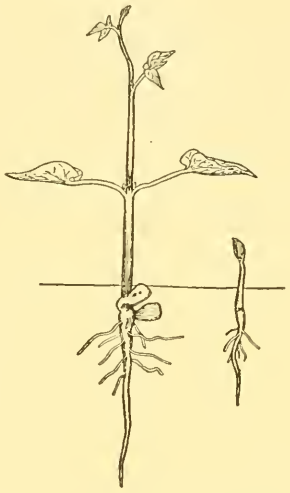


Fig. 117.—Seedlings of *Phascolus*. On the left a normal one; on the right with cotyledons removed at the beginning of germination (*after Osterhout*).

Since during the first stages of its development the young plant is not yet capable of independent nutrition, the presence of such reserve substances is very important for its successful development. The removal of these substances, for instance, by cutting off the cotyledons, results in retarded growth (Fig. 117) and may even cause the death of a plant. Not only complete removal but also a holding back of the reserves of the seed leads to slower growth. It has been shown by Blackman's formula that during the first stages growth proceeds according to the compound interest law. Therefore, the dimensions of a plant are to a considerable degree determined by the amount of the initial "capital," *i.e.*, the quantity of reserve substances and the size of the seed in general. Hence seed size is of profound influence on the further development of the plant. As has been often said, from bigger seeds there usually develop more vigorous specimens (Fig. 118). On the importance of size of seed material is based the practice common in agriculture of grading and selecting the larger grains for sowing.

89. Influence of Temperature on Growth. Thermophilous Organisms.—Like all other vital processes, growth of plants is

to a considerable degree dependent on temperature, being accelerated by a rise, and retarded by a decrease (Fig. 119). Within the range of 0 to 35°, approximately, the influence of temperature obeys Van't Hoff's rule. Namely, a rise of 10° C. nearly

FIG. 118.—Seeds of tobacco grown under identical conditions from small (left), medium (center), and large (right) seeds of the same variety (*after Duggar*).

doubles the rate of growth. But above 35 to 40° C., it begins to decrease rapidly and almost ceases. Thus in growth one can distinguish the same three cardinal points, a minimum, optimum and

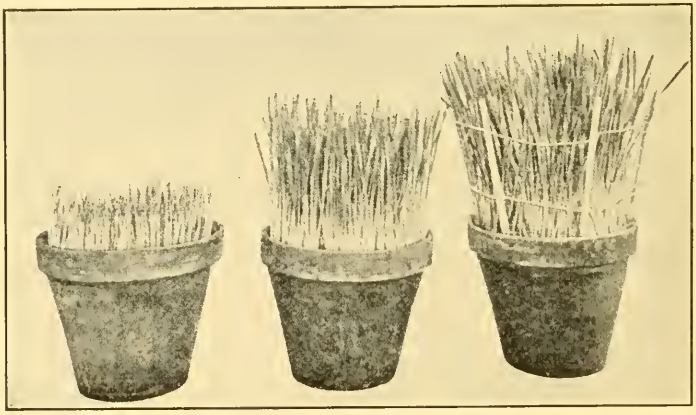


FIG. 119.—Oat plants 8 days old, grown at 8° C. (left); 15° C. (center); and 25° C. (right) (*after Nathansohn*).

maximum, which were pointed out in the discussion of the influence of temperature on assimilation (Art. 14).

In respect to the effect of temperature on growth, the various plants are by no means identical. A distinction may be made

between thermophilous plants with very high cardinal points and plants resistant to low temperatures, but more sensitive to excessively high ones. An absolutely precise determination of these cardinal points for higher plants presents great difficulties, since the influence of temperature is usually intricately connected with the influence of other external factors. An approximate idea of the temperature requirements of different plants may be gained from the table of germinating temperatures for seeds, compiled some fifty years ago by Haberlandt.

CARDINAL TEMPERATURE POINTS FOR GROWTH OF SEEDLINGS

Degrees Centigrade

Plant	Minimum	Optimum	Maximum
Barley, oats, rye, wheat	0 to 5	25 to 31	31 to 37
Buckwheat	0 to 5	25 to 31	37 to 44
Hemp	0 to 5	37 to 44	44 to 50
Sunflower	5 to 10	31 to 37	37 to 44
Maize	5 to 10	37 to 44	44 to 50
Pumpkin	10 to 15	37 to 44	44 to 50
Melon, cucumber	15 to 18	31 to 37	44 to 50

The table shows that not only the absolute position of the cardinal points, but the latitudes of temperature between each, are different. In general, the discrepancy between the minimum and the optimum is always considerably greater than between the optimum and the maximum. The former is usually 25 to 30° C., while the latter does not exceed 5 to 10° C. This seems to indicate that beyond the optimum the injurious influence of high temperature begins to have an influence.

The minimum and maximum points are the temperatures at which growth is checked, but which do not cause the death of the plant. The minimum for life, the so-called ultraminimum, is usually below the minimum for growth, while the maximum for life, ultra maximum, is somewhat above the maximum for growth. Between these points there is a stage at which growth ceases, a peculiar inhibition of the organism. Plants can withstand temperatures in this range without injury, if exposure does not continue too long. A prolonged retardation especially from excessive heat

may allow the development of disease and may even cause the death of an organism.

During retardation there is no growth, but the other vital processes, such as respiration, assimilation, metabolism, etc., still continue. The cause of this check in growth must be looked for in the disturbed harmony between the separate functions of the plant, since growth is a very complex process requiring a precise coordination of all functions. The rate of the various metabolic processes, however, changes in different degrees with variations of temperature. For instance, respiration increases till a lethal temperature is reached, its maximum and optimum coinciding, while assimilation decreases considerably earlier. Therefore, at temperatures exceeding the required optimum for assimilation, consumption will overbalance assimilation and the plant will soon be exhausted. Hence, the optimum temperature for growth is by no means the most favorable one for the general development of a plant. According to the usual definition, the optimal point is one at which growth proceeds most rapidly. But the highest rate of growth is not always the most desirable for the development of a strong and healthy plant. Because of the rapid consumption of food materials, plants which show fast development or, as gardeners would say, have been forced at a high temperature, are usually weaker than those which have developed at a lower temperature. A purely physiological optimum, therefore, indicating the greatest rapidity of development, must be distinguished from the harmonious optimum, producing the most vigorous plants.

It is quite evident that the exact determination of such an optimum presents greater difficulties than the estimation of the physiological optimum. It requires, in the first place, much technical equipment such as several greenhouses where temperatures can be regulated with precision. This has not been available at any of the scientific institutions. Recently, however, the construction of such greenhouses has begun at some of the wealthier American institutions. For the present one must be contented to obtain approximate information from the observation of plants in their natural state. Such observations usually show that the harmonious optimum is not constant for the whole period of development of a plant. It varies continually from germination to flowering and maturity. For most annual plants, at least, a general rule can be established, that in the earlier stages of devel-

opment this optimum lies at a lower point than during the later ones. This coincides with the natural march of temperature from spring to early fall. A temperature too high at the beginning of growth is one of the chief causes of the development of weak plants yielding a poor crop. This commonly results from late sowing. This rule, however, cannot be applied to thermophilous plants of tropical origin.

Certain plants may even show a pronounced need of cold temperature. Such are the winter cereals which are usually sown in autumn and, after a prolonged winter period, produce seed during the following summer. If sown in the spring, they will tiller throughout the summer without reaching the fruiting stage. Formerly it was thought that winter plants require a rest period. Gassner, however, demonstrated that if winter cereals are allowed to germinate at a temperature not higher than 3 to 5° C., and after that are planted in the open, production may be induced in the first year without any intermission in the development of the plants. Thus, the winter cereals may be considered as requiring a cold environment during the early stages of growth. The same may be said of several spring plants, especially certain kinds of oats and vetches, which bear earlier and better fruit when allowed to germinate in cold weather.

Investigations of the temperature requirements of lower plants, notably the saprophytes which do not require light, are of course much easier, and hence more exact data are available.

In this group of organisms, a much greater diversity is encountered than among the higher plants. Here may be found certain fungi, like *Fusarium nivale*, frequently causing considerable damage to winter cereals, and certain thermophilic bacteria, which develop in damp hay and heating manure. The temperature minimum of many of these thermophilic bacteria and fungi lies at 25 to 35° C. At room temperature they commonly die. Their maximum temperature may be as high at 70° C. It is interesting to note that some of these organisms have a very high respiratory rate. Great quantities of organic substances are oxidized, which causes considerable self-heating of hay and manure. In this heating, the consecutive work of various microorganisms may be observed. The process is started by common putrefactive bacteria and molds which raise the temperature to 35 to 40° C. Then, the thermophiles make their appearance driving the heat up to 60 to 70° C.

and supplant their predecessors. The self-ignition of hay which sometimes succeeds self-heating is a purely chemical process of auto-oxidation of the material dried out by a high temperature.

To the thermophilous organisms belong also some of the blue-green algæ of hot springs which, according to certain data, have been found thriving at a temperature of 60 to 70° C., and even of 93° C.

90. Effects of Light on Growth. The Daily Periodicity of Growth. Formative Action of Light.—Contrary to temperature, light is not a factor necessary for growth. Practically all plants can grow in darkness, while some plants, such as the bacteria and many fungi, can complete their whole development in absolute darkness without essentially altering the character of their growth. Higher plants, at least their green organs, usually show in the absence of light a rather marked divergence from their normal form and are deprived of green color. Such plants are said to be etiolated.

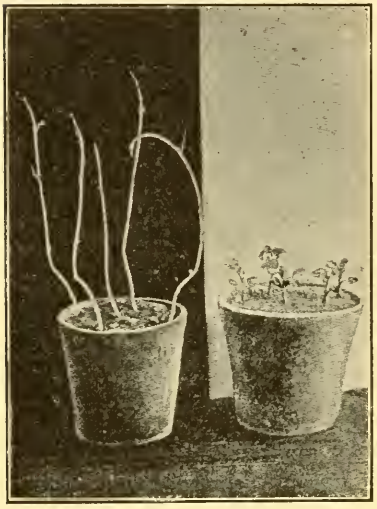


Fig. 120.—Etiolated and normal seedlings of the horse bean.

Etiolated plants differ from normal ones mainly by having excessively elongated stems and underdeveloped leaf blades (Fig. 120). In case, however, the stem does not grow during the first stages of development, a stretching of the leaves takes place. This faculty of elongating its organs in darkness is of great importance in the life of a plant. Finding itself deep under the ground, it stretches its shoots till they have reached light and then begins to unfold its leaves. Anatomically, etiolated plants are distinguished by tissues which are very little differentiated and by the prevalence of primary parenchyma.

There is no direct connection between etiolation and the absence of chlorophyll or the shortage of nutrition. Stretching in darkness is observed in many fungi, for instance *Mucor, Pilobolus, Coprinus,*

etc., while very often no sporangium (*Mucoraceae*) or pileus (*Basidiomycetes*) is formed, or else are underdeveloped. Elongation in darkness is likewise pronounced with sprouts of the potato which are abundantly supplied with nutrient substances from the tuber. In spite of numerous attempts to explain the causes of the peculiar alterations in form of etiolated plants, our knowledge of the subject remains very scanty. Growth in an atmosphere saturated with water vapor also leads to poor development of leaves and elongation of stems as well as retarded differentiation of tissues. Hence, many authors, Palladin, for instance, are inclined to explain, in part at least, changes in the form of etiolated plants by curtailed transpiration in the absence of light (Art. 51). Recent observations, however, have shown that even the shortest daily exposure to light, 5 to 10 min., proceeding, moreover, from a rather weak source, is sufficient to induce etiolated plants to develop a normal form. This occurs in spite of the fact that in so short a time transpiration is not able to change to any extent, nor assimilation to begin, since no chlorophyll is formed. It is probable that under the influence of light there arise in the plant some substances of the nature of hormones, which lead to changes in the character of growth of leaves and stems. This assumption is confirmed by the fact that violet and ultra-violet rays are of the greatest effect in changing form, while the red part of the spectrum, which increases assimilation and transpiration to the highest degree, in this case produces the least effect (Fig. 121).

Light has also a considerable influence on the rate of growth of plants. It retards growth in a definite way, and the higher its intensity, the greater the retardation. Moreover, under the influence of light, plants pass more rapidly through the grand period of growth, the period of elongation terminating very early; before the growing organs have had time to elongate. Consequently, shoots that have developed in strong light are always shorter than those grown in shade.

The behavior of the seedlings at the moment they have pushed their way through the earth and emerge into light, is very interesting: their growth is instantly checked. This delay affects equally all parts remaining in the soil. Under the influence of the light, there evidently arises in the tip some growth-delaying stimulus which is transmitted to the underground parts. Some authors

hold that this stimulus is a special growth-checking hormone diffusing from the top downward. Others think that light delays the manufacture of the growth-promoting substance necessary for the elongation of the cells (Art. 86).

The dwarfing effect of light on the aerial organs is particularly marked in high mountains, where, because of greater transparency

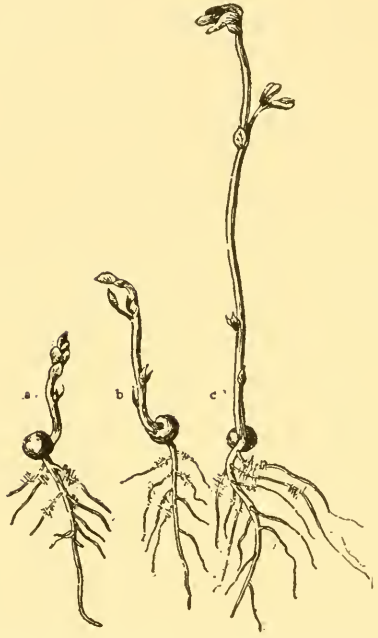


Fig. 121.—Seedlings of peas grown for an equal period (*a*) in white, (*b*) blue, and (*c*) orange light (*after Duggar*).

Fig. 122.—*Betonica* plants, *P*, grown in the valley, and *M*, upon a mountain (*after Bonnier*).

of the atmosphere, solar light is considerably richer in ultra-violet rays than in valleys. Alpine plants, as the investigations of Bonnier have shown, are always of short stature. And when common plants of the valley, for instance the dandelion, or Betonica, are grown in high mountains, they assume the characteristics of alpine plants (Fig. 122). The retarding influence of

light on growth is so great, that it creates a definite daily periodicity. During the night plants grow more rapidly than during the day, in spite of the lower nocturnal temperature. Only at a very sharp drop of temperature the accelerating influence of absence of light fails to overcome the retarding influence of lowered temperature, a condition leading to stunted plants as exemplified by the vegetation of the tundra. Low temperatures at night have an influence even on the growth of alpine plants, adding their effect to the influence of bright light in producing increased dwarfing.

The checking effect of light on growth manifests itself markedly in many microorganisms which even in diffused light develop poorly and die in direct sunlight. Upon this fact is based the sterilizing value of light, so well known in hygiene and medicine. Gloomy, damp, and badly aired rooms are the breeding places of disease germs. Light is also an excellent disinfectant of the human skin, which explains, in part at least, the beneficial value of sun baths. In this respect, of the greatest importance are the ultra-violet rays. Hence in recent years light from quartz mercury-vapor lamps, which is rich in ultra-violet rays, has been used as a substitute for sunlight.

In prolonged experiments with green plants, the growth-inhibiting influence of light may be masked by its indirect effect. Being a source of the necessary energy for the production of organic matter, light promotes nutrition and consequently better growth of plants. Therefore, in well-lighted places, plants generally develop better than in shaded ones. Thus, for instance, plants will grow better in a room when kept near a window than when farther back from it. The light requirements of the various plants vary greatly, as has been seen in Art. 12. A light intensity which is insufficient for some plants may be excessive for others. The anatomical and morphological differences between light and shade plants, considered in the same paragraph, are due more to the indirect than the direct effect of light. The considerable increase of transpiration in light is of particular significance in this respect.

91. Influence of Moisture on Growth of Plants.—The amount of moisture in the environment has a profound effect on the growth of plants. The greatest increase in volume of the cell occurs, as has been seen, during the stage of elongation. It is largely due to the water which enters the cell. This stage therefore can be normally attained only when water has an uninter-

rupted and unhindered access to the growing cells, which is possible only when it is used at a comparatively slow rate. With deficiency of water or an accumulation in the soil of substances inhibiting water supply (Arts. 45 and 46), the stage of elongation terminates too early and differentiation begins before the cells have reached their full size. The result will be a plant with smaller cells and shorter growth.

A too rapid expenditure of water leads to the same consequences. Leaves that transpire fast cannot obtain water rapidly enough from the soil and will draw it from the growing organs. Under these conditions, the expanding cells will be disturbed most, resulting in diminished size. The drawing of water by the older leaves from the growing ones may explain the law of Zalensky already discussed in Art. 58, according to which the upper leaves have always smaller cells and show the fundamental xeromorphous structure. This may be chiefly due to an insufficiency of water supply for the growing organs.

In cereals to which belong the most important crops, such as wheat, rye, barley, and oats, the developing stem shows a peculiar growth distribution. First, only meristematic growth takes place in the stem, and only after all its internodes, as well as inflorescences, have been laid down, does elongation, or the so-called shooting, set in. During this period, the plant is especially in need of an ample water supply for the growing parts and, hence, is sensitive to its deficiency. Field observations have shown that an insufficient rainfall during the period immediately preceding shooting greatly reduces the yield of cereals.

Experiments have demonstrated likewise that the same amount of wilting of plants will produce quite different effects if it occurs at different periods of their development. Wilting of tillering plants, *i.e.*, during the period of meristematic growth, or in the time of blooming, coinciding with the stage of internal differentiation, is less detrimental than wilting at the time of shooting. Hence, in respect to moisture supply, this stage of development is known as the critical period (Brounov).

The most favorable conditions for growth are related to an ample moisture supply in the soil as well as the atmosphere during all periods of development of a plant. Consequently, the greatest vigor and the highest rate of growth are attained by the vegetation in the moist and warm tropical countries. A constant heavy

moisture supply, however, may have certain unfavorable consequences. The development of protective tissues, the cuticle and cork, is weaker in moist air and hence the plant is more exposed to the attacks of parasitic fungi, whose spores germinate readily in a moist medium. Moreover, in many plants air with a high moisture content retards the ripening of fruits. Excessive moisture in the soil makes it swampy (Art. 46), which is detrimental to most plants. Most mesophytes and especially xerophytes develop better in moderately moist habitats.

92. Effects of Chemical Stimulants on Growth.—In considering the influence of various external factors on growth, it is necessary to keep in mind the following points: Growth is the result of complex chemical and physical processes, proceeding in the cell under continuous expenditure of energy which is liberated through respiration. Thus growth is closely connected with respiration and ceases as soon as a plant is transferred to an oxygen-free medium. Other external influences, with the exception of temperature which regulates the rate of all vital processes, and water, by which the material for enlargement of cells is supplied, do not act upon growth directly, but only indirectly, by producing certain deviations of the processes on which growth depends. These alterations are due to the plasticity of the factors involved. Their characteristic peculiarity is the lack of relationship between the amount of the acting stimulant and the effect produced. Sometimes a feeble stimulus may produce a strong effect and, sometimes, quite the contrary, a strong irritation is almost without influence on growth. The effect depends largely on the nature of the stimulating agent, and on the degree and the direction of deflection of the normal course of growth. The phenomena of irritability are often compared to disturbances produced on a working machine. Sometimes a feeble touch, but one that may cause to turn some important lever, may completely change the movements of the machine, while a heavy shock applied to other parts may produce no effect. Since it is not known, as yet, what levers in the living machine, the protoplasm, respond to the action of certain stimuli, it is not surprising that these phenomena appear to be very complex and that very contradictory experimental results have been secured.

The effect of one of the physical stimulants, light, has been discussed already (Art. 90). The influence of other stimuli, chiefly of

a chemical character, will now be considered. The foremost place must be assigned to the cations and anions. It has been noted (Arts. 25 and 26) that the presence of a definite combination of ions in the solution entering into the cells is a necessary prerequisite of plant growth. In a study of the indispensable individual ions, it is, however, very difficult to distinguish their growth effect in the building up of new particles of living matter from their action as specific stimuli. Hence, there is more information on the influence exerted on growth by the less necessary and even poisonous substances. By poisonous substances are understood chemicals which even in small doses inhibit growth and in considerable quantities will kill a plant. Such, for instance, are the salts of heavy metals, copper, lead, silver, etc.; as well as many organic substances like ether, chloroform, toluol, etc. Certain metabolic products of an organism also may be poisonous, such as the organic acids, notably oxalic acid.

A detailed study of the influence of poisonous substances on growth usually shows that not only the severity of their action, but its character depend on their concentration. When applied in very weak doses, the majority of even the most poisonous substances will not depress, but stimulate, growth. For purposes of calculation, assume a solution of 1 gram-molecule per 100,000 liters of water as unity, then phenol, carbolic acid, for example, in a concentration of from 100 to 200 will stop the growth of plants in water cultures, but it will have a stimulating effect in concentrations of 4 to 8. Ethyl alcohol, a weaker poison, stimulates in a concentration of 25 to 75 and checks in concentrations of 2700 to 7500. Some poisons, as copper salts, do not produce a stimulating effect.[1]

It is interesting to note that the effect of a poison depends also on the properties of the medium in which a plant is growing. The poisonous quality of many substances markedly decreases in sand cultures, while in soil the plant is able to endure several hundred times as large a dose as in water culture. This is due to the adsorption capacity of the soil, which binds the poisonous substances on the surface of its particles.

The action of poisons does not differ essentially from that of substances such as the mineral salts, since in strong concentrations these too have an inhibiting effect on growth. But they

[1] In some soils of the United States small additions of copper salts have markedly stimulated plant growth.

can also stimulate it, if applied in weaker dilutions. The difference must therefore be considered to be rather quantitative than qualitative, and only substances which inhibit growth in very weak concentrations should be regarded as poisonous. The action of all cations in a pure state is also poisonous. Naturally only those solutions are good nutrients for plants in which the ions are in equilibrium. An especially poisonous influence is exhibited by the H^+ ion. For the majority of plants, with the exception of some molds, a considerable acidification of the medium is lethal. The different plants are not equally susceptible to the concentration of hydrogen ions and this (Art. 32), explains to a considerable extent the distribution of plants in relation to soil acidity.

The mold fungi are especially convenient objects for the study of both the stimulating and the poisonous effect of various substances. In his classical work on the conditions of growth of *Aspergillus niger,* Raulin has shown that the yield of this fungus depends not only upon the presence in the solutions of definite mineral and organic compounds serving directly as nutrients, but also on the presence of special stimulants, which can alter growth considerably. Thus, for example, the addition of very small quantities of zinc sulphate (0.0005 per cent) caused a two to threefold increase of the yield. The same experiments have been confirmed by later work. In growing microorganisms, the possibility of the stimulating influence of organic and inorganic compounds is now always taken into account. These observations have lately found application in agriculture. By his investigations of the distribution and rôle of manganese salts in plants, Bertrand laid the foundation of the doctrine of the so-called complementary or catalytic fertilizers. These fertilizers, chiefly manganese salts, produce better crops not as direct nutrient substances, but as stimulants of growth and development. The rôle played by iron in plant life also appears to be largely of a catalytic nature.

Catalytic fertilizers have not found wide practical application. The addition of manganese sometimes produces an increase in yield, while at other times it remains without influence. The conditions under which it may be definitely effective have not been established as yet. Growth stimulants have recently received a new emphasis through the efforts of the Bulgarian professor, M. Popoff, who is of the opinion that it is not absolutely necessary that the stimulant should act continuously upon the plant during

its development. In order to increase growth it is sufficient to stimulate the germinating seeds. For this purpose, Popoff uses 3 per cent solutions of magnesium chloride and sulphate and manganese sulphate and nitrate, as well as mixtures of these salts, and a number of organic compounds, especially tannin and various narcotic and antiseptic substances. The seeds are soaked in these solutions for several hours and then sown in the usual way. According to Popoff, as a result of such a treatment growth has been accelerated, the size of the plants has increased, and considerably larger crops have been obtained. In some of his experiments this increase has amounted to as much as 50 to 100 per cent.

Popoff's experiments have attracted a great deal of interest, since by stimulation of seeds a considerable increase in yield may be obtained at little expense. But most investigators have not been able to confirm his results, especially under field conditions. Stimulation of seeds is a considerably more complicated affair than it appeared at the outset. The conditions under which the experiments succeed have not as yet been determined.

In close relationship with the phenomenon of stimulation are methods of forcing (Art. 87). In forcing, both chemical and physical agents are applied as stimulants. As in the case of seeds, so here the stimulus once given leads to changes in the character of development of a plant which continue for some time.

Under chemical stimulants are included also the so-called hormones. They are formed by the organism itself, and produce changes in the rate and direction of chemical reactions of the cells and organs, thus altering the very characters of growth and development of an organism. The action of hormones was first discovered in animals in which they are formed by special ductless glands, named glands of internal secretion. Such are the suprarenal capsules, the thyroid gland, sexual glands, etc. The hormone of the suprarenals, adrenalin, has been obtained in pure state and its chemical composition has been established. Plant hormones are more difficult to determine. The best studies of their activity are in connection with increased division and the growth of cells on the surface of healing wounds.

It has been demonstrated by Haberlandt that the increased division of cells results not from mechanical injuries nor an increased access of air to the bared cells, but from altered chemical action of the cut cells. Before disintegration they seem to release some sub-

stances which stimulate division in the neighboring cells. If a freshly cut surface of plant tissue is immediately washed with water, then there seldom occurs any division or growth in the cells adjoining the wound. But if the wound is smeared with a paste consisting of minced tissues of the same organism, then division increases considerably (Fig. 123). The substances thus stimulating division are called by Haberlandt "wound hormones."

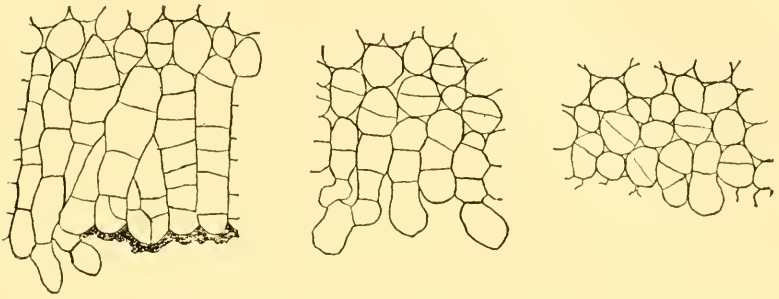


FIG. 123.—Multiplication of cells on the surface of a wound of kohlrabi. Wound not washed (center); washed with water (right) smeared with a mass of minced kohlrabi (left) (*redrawn after Haberlandt*).

They probably are specific, since material from the tissues of another plant produces no effect. Another instance of a plant hormone is the "growth-promoting substance" recently detected by Wendt (see Art. 83), which seems to be an indispensable condition for the elongation of plant cells.

The hormones are considered as playing an important rôle in the regulatory movements of plant organs. They may be likewise associated with the reproduction of plants. In the following chapter examples of their effect will be given.

CHAPTER XI

THE MOVEMENTS AND SENSITIVITY OF PLANTS

93. Plant Movements and Their Mechanism. Sensitivity.—Since plants, especially the higher ones, are firmly fixed in soil by means of a root system, they are not able to change their position. Hence it is often believed that they lack entirely the capacity of movement. This, however, is erroneous, for, though the plant as a whole is incapable of moving from place to place, its separate organs may change their position, though at a very slow rate, in dependence on alterations in the external environment and internal conditions. Thus, plants which are placed near a window turn their leaves toward the light. A fallen stem, or one which has been placed in a horizontal position, usually raises its tip. And many flowers open in the morning and close at night.

The movements of plant organs, similarly to those of animals, are effected by definite external stimuli. Each of these changes in position is a response to some alteration in the environment. But in order to produce a responsive movement every stimulus first must be perceived and must cause a definite state of excitation at the place of perception. If the region of perception and the region where the movements take place are separated, then the transmission of excitation must occur. This is analogous to the complex phenomenon which is known as a "reflex" in animal physiology. Hence, one may also regard movements in plants as being conditioned by reflexes, though much simpler ones than those present in animals. This simplicity is due to the fact that plants do not have special tissues for the transmission of excitation. They have no nervous system. Moreover, they are devoid of sense organs of a complex structure, such as are possessed by animals. The extremely slow rate at which excitation is transmitted in plants, usually 0.0003 to 0.006 cm. per second or at most 1 to 10 cm. per second in *Mimosa*, is also due to the absence of a nervous system. While in the frog, for example, excitation is conducted at a rate of 28 m. per second and in man 120 m., plant movements are rela-

tively slow. The turning of a leaf towards light or the raising of the tip of a prostrate stem require several hours for their completion. Only in exceptional cases, as, for instance, with the catching apparatus of insectivorous plants or the leaves of *Mimosa* and the leaflets of *Oxalis*, are the movements rapid enough to be observed.

This slow rate of movement and low degree of development of perception and transmission, as well as the relatively unimportant rôle of movement in general in the life of the plant, are, however, by no means indicative of a lower type of organization in plants. The lower plants, algae in particular, possess a more highly developed capacity for movement than do the higher plants. Many of them, as for instance, *Chlamydomonas* and the diatoms, are no less mobile than animal organisms of a correspondingly low organization, such as the infusoria and the flagellata. As the organization of plants becomes more complex, their capacity for movement seems to be gradually reduced. This change is closely connected with the character of plant nutrition and with the capacity of photosynthesis, which, as we have already mentioned, is characteristic of plants. Since plants receive all the required nutrients from their environment by means of diffusion and osmosis and are able to form the most complex organic compounds from these simple substances, they are not forced to search for food and, therefore, stand in no need of mobility. They have, therefore, almost lost this capacity in the course of their evolution.

The mechanism of movement in plants is also characterized by simplicity. There are no special muscular tissues in plants. Most of their movements are realized by means of an unequal rate of growth on the different sides of a bending organ. The side which for one reason or another develops more rapidly, thus becomes more convex, while the other side, where growth is delayed, becomes concave and the organ curves in the direction of the side where growth is the least. Such movements of course may be observed only in organs whose growth is not yet completed. They, therefore, are called "growth movements." One may distinguish two types of such movements: *tropistic* curvatures, caused by a unilaterally acting stimulus, such as light or the force of gravity, and *nastic* curvatures, caused by a stimulus affecting the plant from all directions, as for instance, a change in temperature or humidity.

Besides growth movements, which are typical of plants, one may also observe another type of movement resulting from changes in turgor pressure within the cells. These are more rapid than growth movements. Their best-known example is the movement of *Mimosa* leaves. Not being subject to growth, they may be repeated, and hence are called alternating movements. Only a small number of plants exhibit this phenomenon, while growth movements may be observed in all plants. Still their study has been of very great interest, as in their nature they are nearest to the movements of animals and are conditioned by a comparatively rapid transmission of irritation.

Some lower plants, particularly those having only one cell, exhibit free locomotion of the whole body by means of special organs of movement, such as the cilia or flagella. These movements in no way differ from those of lower animals and hence they will not be discussed here.

Likewise, no consideration will be given to such changes in position of plant organs as are not caused by physiological, but by purely physical processes, as, for instance, the opening of many fruits during drying (geranium, vetch, *Oxalis*) the curling of the awns (beards) of wild oats and *Stipa*; the bending of branches of trees in winter, due to changes in temperature; the splitting of certain juicy fruits (*Impatiens*), etc. Though many of these movements play an important part in the life of a plant and are frequently connected with a rather intricate mechanism, still it is impossible to regard them as manifestations of the life activity of a plant.

94. Phototropism.—It has been noted that light is the most indispensable factor for the existence of green plants. It provides them with energy required for the decomposition of carbon dioxide. It is but natural, therefore, that plants should show a high degree of sensitivity to light and should respond effectively to all changes of the direction of light rays falling upon them.

The capacity of turning toward or from the source of light is known as "phototropism." It was formerly called "heliotropism" (from the Greek word *helios*, the sun), as daylight was then chiefly used for experimental purposes. Young growing stems usually bend in the direction of light, this reaction being known by the term "positive phototropism" (Fig. 124). The curving of plants away from the source of light is called "negative phototropism,"

while the capacity of the plant to keep its organs perpendicular to the direction of light rays is known as "diaphototropism." As a rule, organs of a dorsiventral structure, such as the leaves, the thallus of the liverworts, are diaphototropic, while axial organs of a radial structure exhibit either positive or negative phototropism.

Not all the organs of a plant are phototropically sensitive. This is a characteristic feature only of the aerial parts of plants. The underground organs, such as the roots and the rhizomes, which normally grow in darkness, very often show no reaction to

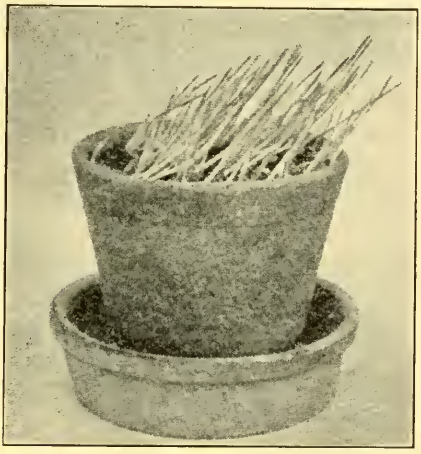


FIG. 124.—Seedlings of oats bending towards light (*after Nathansohn*).

light. Some roots, though, as those of mustard and other crucifers, are negatively phototropic.

The capacity of bending toward or away from light is not permanent for every organ. It depends on the intensity of light. In too strong light positive curvatures frequently change to negative ones. By selecting various intensities of light, it is possible, therefore, to make the same shoot bend in succession towards and away from the source of light. It is also possible to find a certain intensity of light in which the positive effect will be neutralized by the negative, and the plant will then appear to be entirely insensitive to light.

Phototropism plays an important rôle in the life of plants. Because of it, leaves, which require light, assume a position that is

most favorable for the utilization of this source of energy. They usually spread perpendicularly to the direction of the incident rays (Fig. 125). Owing to phototropic movements, the leaves of many plants form the so-called leaf mosaic, which is particularly conspicuous in shady plants, for example, the ivy (Fig. 126). In general, phototropic phenomena are more distinct with plants growing indoors or in the shade than in those grown in open spaces.

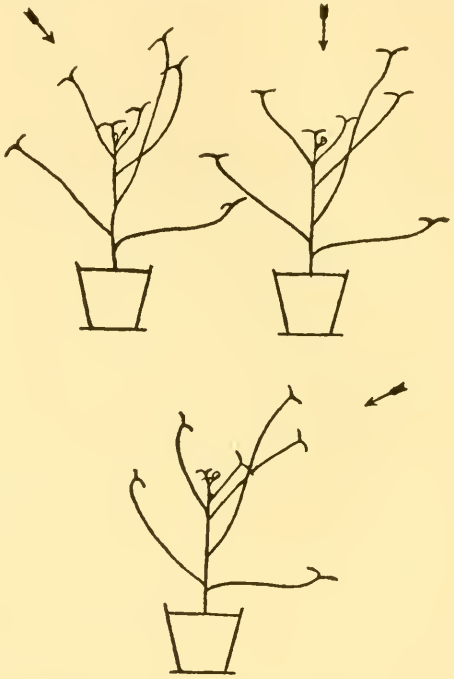


FIG. 125.—A geranium adjusting its leaves perpendicular to the direction of light (*after Lubimenko*).

This is due partly to the fact that in very strong light phototropic sensitivity is somewhat lowered. It may be caused also by the diurnal and continuous change in direction of sunlight. Some plants, however, show so rapid a reaction to an alteration in the direction of light that they follow the motion of the sun. Good examples of such types are the sunflower and bur marigold (*Bidens*).

Phototropic sensitivity is in no way connected with the presence of chlorophyll. Sporangia and the fruit bodies of many fungi also manifest phototropic curvatures. Moreover, etiolated seed-

lings are always more sensitive to light than are green ones. In general, it has been found that growing plants in light decreases their phototropic sensitivity in proportion to the intensity of light,

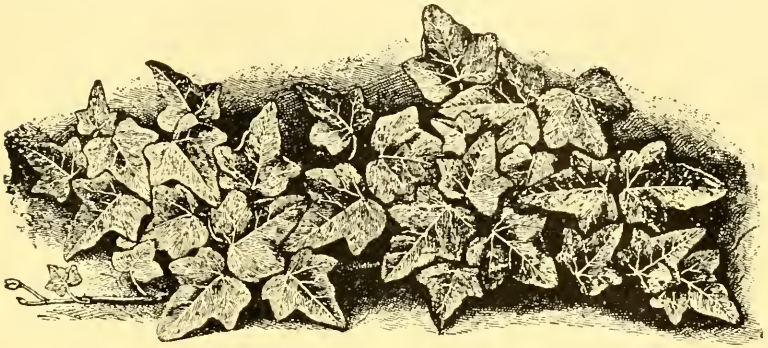


FIG. 126.—The leaf mosaic of an ivy (*after Lubimenko*).

therefore, plants in open spaces are less sensitive to light than those growing in the shade, while the most sensitive are those which have grown in total darkness. The latter are, as a rule, used for precise phototropic experiments. These experiments are carried out in phototropic chambers, tight boxes having their interior painted black and admitting light through a small opening in one of the walls (Fig. 127).

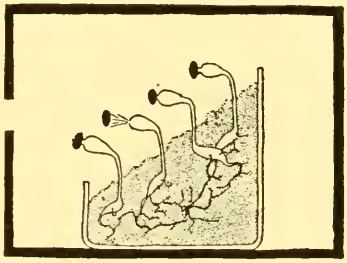


FIG. 127. — Sporangia of *Pilobolus* turned towards the openings of a phototropic camera (*after Smith, et al.*).

Similar to all responses to irritation, the phototropic process consists of four phases: (1) the effect of the stimulus (in the present instance, of light) on the cells; (2) the creation of excitation in the cells; (3) the transmission of excitation to the region of reaction; and (4) the response itself, which in this instance consists in the formation of a curvature.

As a rule, only the first and the last phases of the process may be observed. But Darwin (1880) succeeded in proving that in cases of phototropic curvatures it is not merely a question of the direct effect of light on the bending parts. In a number of later investigations, particularly in those by Rothert (1894) a definite subdivision of the process into separate phases was attained.

The seedlings of some cereals, particularly those of sorghum and millet (*Setaria*), are most convenient objects for such experiments. These seedlings consist of a rather long hypocotyl bearing a comparatively small plumule (cotyledon) which contains the first true leaf. If the shoot is placed in a phototropic chamber, the hypocotyl will form a distinct curve and the tip of the plumule will be directed toward the light (Fig. 128). At first, the curvature is formed directly beneath the plumule, but, later, as further growth of the hypocotyl takes place, the curvature is somewhat lowered. By means of very simple experiments it may be shown easily that the plumule is the organ which perceives light, while the hypocotyl merely responds to the excitation transmitted from the plumule. If an opaque hood made of black paper or tinfoil is placed over the tips of the shoots, so as to cover the whole of the plumule, no curvatures are formed, notwithstanding the fact that the hypocotyl remains exposed to light. On the other hand, if the hypocotyl is shaded from light while only the plumule remains exposed to it, curvatures are formed as distinctly as when the whole of the shoot is exposed.

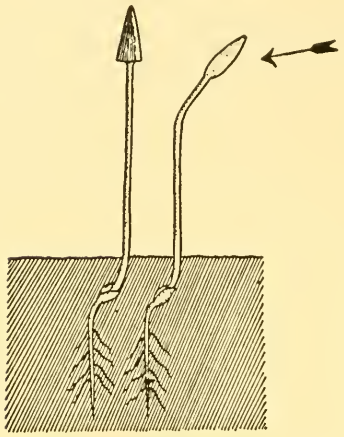


FIG. 128.—Seedlings of *Setaria*. On the right, one bent towards light; on the left, one of the seedlings has remained upright. Its plumule has been covered with a non-transparent cap (*after Holman and Robbins*).

The mechanism of phototropic curvatures consists in a more retarded growth of the exposed side of the stem, as compared to the growth of the shaded side, thus the lighted side becomes shorter and the entire organ will curve. Comparing this fact with the long-known phenomenon of delayed growth caused by the effect of light, many authors, beginning with De Candolle, attributed this bending of plants towards light to the direct retarding effect of light on the growth of the exposed side. But considering the experiments which have demonstrated that perception is localized in the tip of the shoot, which, however, is not directly subjected to curving, the foregoing explanation has to be modified

as follows: a unilateral exposure to light causes certain inhibitory substances to appear in the organ of perception, which descend to the growing zone and produce a delay of growth on the exposed side.

Recent experiments have shown that these inhibitors are definite substances and that the transmission of excitation from the zone of perception to the zone of reaction proceeds by diffusion. Boysen-Jensen's experiments may be considered as a definite proof of this fact. Though his results were at first seriously doubted, later they were fully confirmed by a number of other investigators (Paal, Stark, Wendt, Cholodny, and others). These experiments have shown that excitation caused by a unilateral exposure to light of the tips of shoots of oats which possess a peculiarly high sensitivity, and therefore usually serve as objects for phototropic experiments, is transmitted downwards to the zone of reaction not only through the living tissue, but through a thin layer of gelatin as well. The tip of the shoot serving for this experiment is first cut off and then fixed in its former position by means of gelatin. Further experiments have shown that the tip may not only be attached to the same shoot from which it was removed, but to another previously decapitated shoot as well, and that this is also followed by the formation of a curvature even in the case when the shoot to which a foreign "head" has been affixed was not subjected to unilateral exposure to light (Fig. 129). Moreover, it is possible to attach the removed tips of oat shoots to the decapitated shoots of other cereals, such as wheat, barley, and rye, and still the excitation produced in the tip will cause the formation of a curvature, though a somewhat less marked one, in the lower parts of the shoot which are alien to them. Even the root tip of maize stuck to the coleoptile of oats, having lost its phototropic sensitivity as a result of decapitation, enables it to react on unilateral illumination.

Excitation, however, is not transmitted through a layer of mica or tinfoil. This leads us to believe that we have to deal with the phenomenon of diffusion and not with the transmission of an electric charge.

If all the data concerning the physical nature and the manner of the transmission of phototropic stimuli are correlated with the dependence of the rate of growth (Art. 86) on the amount of the growth hormone manufactured by the stem tip or the coleoptile, the following mechanism of phototropic curvatures (Cholodny,

Wendt) may be observed. Under the influence of the stimulus of light, the total amount of the growth hormone in the sensitive apex, as well as its distribution, is altered. As the exact quantitative determinations by Wendt have shown, light in itself decreases the amount of the growth-promoting substance in the apex. This explains the delaying influence of light on growth, discussed in Art. 90. Moreover, under the influence of a unilateral illumination there takes place an electrical polarization of the cells, which produce and transmit the growth-promoting substance. The illuminated side receives a negative, the shaded side, a positive charge. Under the influence of this polarization, the current of growth-promoting substance is shifted to the shaded side. The cells of this side elongate more rapidly and more strongly than do those of the opposite side and as a result a curvature of the seedling towards the light appears. As the growth-promoting substance is universal and not specific for every plant, it is understandable why an alien tip may induce almost the same curvatures as the proper one. Other authors (Boysen-Jensen, Stark, Gradmann), however, do not accept this idea and suppose that under the influence of light there arise special growth-checking hormones which differ from the growth-promoting substance.

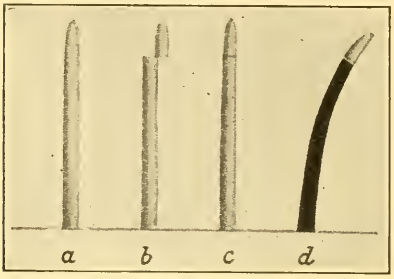


FIG. 129.—Sprouts of oats: *a*, before the experiment; *b*, decapitated; *c*, the removed tip is attached by means of gelatin; *d*, phototropic curvature of a sprout with a fixed tip, the growing side of which is shaded (*after Molisch*).

A similar transmission of excitation takes place in many leaves. As a rule, the leaf blade is the organ of perception, while the petiole or the pulvinus is the seat of movement. In leaves, however, the process, is much more complex, as their adjustment to a position parallel to the direction of light rays is not merely attained through unequal growth, but through complex twisting curvatures.

Until quite recently much discussion has centered around the question as to precisely what is perceived by the sensitive organs of a plant: the direction of light or the difference in the intensity of illumination to which the two sides of an organ are subjected. The question may be considered as finally solved in favor of the

second supposition by Buder's (1920) recent investigations. By means of a very clever device, which he calls a light probe and which consists of a small inverted glass funnel with the inner walls coated with silver and the end bent sideways (Fig. 130), Buder succeeded in illuminating from within the hollow plumule of oats, in which he observed a curvature toward the illuminated side, though the direction of light in this case was reversed as compared to the usual one. In experiments carried out in diffused light, however, it is impossible to take into consideration a definite direction of light rays.

Not all the rays of the solar spectrum produce an equal phototropic effect. Red rays, as a rule, are least effective. A gradual moving towards the blue end of the spectrum shows an increased effect which attains its maximum in the indigo-blue rays (465 $\mu\mu$) and then again gradually decreases towards the ultra-violet region. Thus, one finds here a certain parallelism between the delay of growth and the phototropic effect.

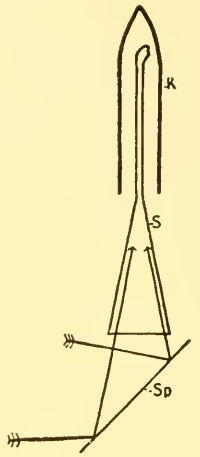


FIG. 130.—Diagram of the light probe. Arrows indicate the direction of light rays (*redrawn after Buder*).

If a plant is subjected to unilateral illumination from any source of light for a short period or before the reaction has taken place, the curvature will nevertheless be formed after a certain period, called "reaction time," has elapsed. The time of illumination required to produce the reaction, is called the "presentation time." Naturally, the length of presentation is directly dependent on the intensity of illumination. The weaker the light, the longer the time of presentation, the product of these two quantities remaining constant. Thus, for instance, Blaauw obtained the following figures for seedlings of oats:

Light Intensity, Meter-candles	Time of Presentation	Amount of Light, Meter-candle-seconds
0.00017	43 hr.	26.3
0.00085	6 hr.	18.3
0.00477	1 hr.	17.2
0.0898	4 min.	21.6
5.456	4 sec.	21.8
1,902.0	0.01 sec.	19.0
26,520.0	0.001 sec.	26.5

The data indicate that the amount of light required to produce reaction is a constant quantity, while the limits of fluctuation shown by light intensity are very great indeed. This important deduction is named the "law of the quantity of stimulus." Further research has shown that this law can be applied not only to phototropism but also to many other phenomena of stimulation. Moreover, in human physiology the same law is known to hold true for the reaction of the human eye to light.

95. Geotropism.—One may observe in higher plants a definite orientation of their organs in respect to the vertical line. The chief axis of a plant, its stem and main root, acquires a vertical position, the stem growing upward and the root downward. The lateral axes of the first order are situated at a certain acute angle to the stem or root, the axes of the second order are again situated at a certain angle, and so on. Leaves, on the other hand, providing they are equally illuminated on all sides, are usually adjusted in a horizontal plane. This distribution is usually characterized by great regularity, particularly in plants of the more primitive types, such as, for instance, the conifers. The regular colonnade formed by a forest of pines and the strictly parallel rows of sunflower and cereal stems are pictures familiar to all, as are also the strictly vertical position of the trunks of gigantic cacti (Fig. 76).

This regularity in the distribution of the axial organs of plants is naturally attributed to the force which always acts in a strictly vertical direction, namely to gravity. The capacity of the plant to acquire a definite position with regard to the vertical line, therefore, has been called "geotropism." As in the phenomenon of phototropism, three things are distinguished—positive geotropism of the roots, which grow downward; negative geotropism of stems, which grow upward; and transverse or diageotropism of leaves, which remain in a horizontal position. A definite proof that gravity is the determining factor in geotropism was supplied by Knight in the beginning of the nineteenth century. He succeeded in solving this very difficult problem by means of an ingenious procedure. The difficulty of finding sufficient proof in this instance was due to the fact that, because of our existence on the earth's surface, we are unable to escape the effect of the force of gravitation, and therefore cannot apply so simple a method as, for instance, the protection of a plant from light, which is used in studying phototropism. Not being able entirely to remove the

objects of his study from the effect of gravity, Knight nevertheless succeeded in discovering a simple method of avoiding its unilateral effect. He placed the seedlings of various plants on a small wheel which was rotated in a horizontal plane. This wheel was kept in motion by means of a stream of water obtained from a brook, while the seedlings, being dipped into the water at every rotation of the wheel, were prevented from drying out. With each turn of the wheel the shoots went through all the positions, from the normal vertical to the horizontal position, then to the inverted vertical, then back to the horizontal, but with a reversed situation of the top and root ends, and finally returned to their original position. This was repeated over and over again. As a result of such continuous motion, these plants no longer had any upper or lower parts. The direction of the force of gravity being continuously changed, the shoots grew in any direction which the investigator chose to give them.

Knight's wheel, so called by the name of its constructor, is rotated at rather high speed. This not only helps to prevent the unilateral effect of the force of gravity, but also creates a somewhat complicated situation, by introducing the effect of centrifugal force. Hence in order to be able better to study the consequences resulting from the removal of the effect of gravity, Sachs modified somewhat Knight's method of procedure by substituting the wheel by a klinostat. By means of this device a rather slow, but very regular rotation is attained, without the development of any appreciable centrifugal force (Fig. 131). The essential part of the apparatus consists of a powerful clock mechanism which is attached to an axial rod. This axis may be adjusted in any desired direction and set to motion at the required speed—most frequently from one to ten rotations per hour. This speed has been found to be sufficient to remove the unilateral effect of the force of gravity, so that it has no time to become fixed and to produce the reaction of curvature in a definite direction.

If a seedling, for instance, of pea or lupin, is placed horizontally, it may be observed that after a certain period, usually a few hours, its stem will turn upwards and its root downwards. If these organs are previously marked at certain intervals with India ink, as is done for the purpose of studying the rate of growth (Art. 85), then it will be noted that the greatest curvature occurs in the region of the most rapid elongation. The fully grown parts

do not manifest any tendency to curve, remaining in the same posi-

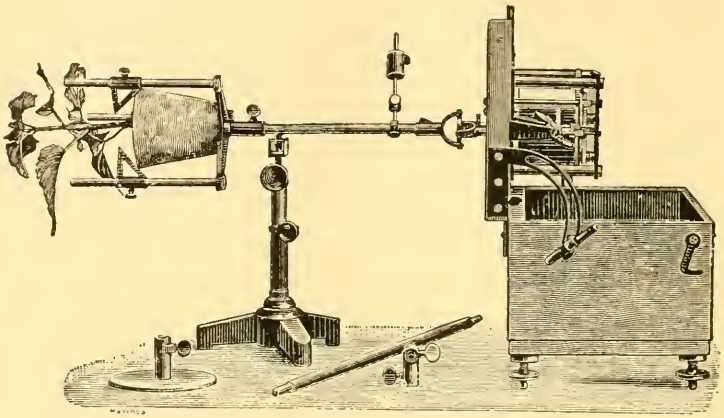


FIG. 131.—A klinostat of Pfeffer's construction (*after Benecke-Jost*).

tion in which they had been placed (Fig. 132). This method is an evident proof that geotropic curvatures are connected with growth, the fully developed parts of a plant having lost the capacity of forming curvatures.

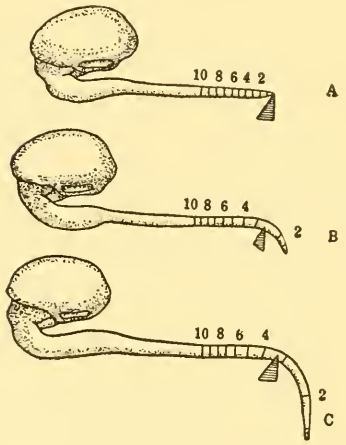


FIG. 132. — Geotropic curvatures of bean roots in humid air. *A*, the original horizontal position; *B*, 7 hr. later; *C*, 24 hr. later. The dark triangle is a paper mark fixed on glass (*after Saehs*).

A seeming exception to this general rule are the culms of cereals, which are capable of rising after "lodging," by forming a bend just above the node (Fig. 133). This phenomenon, which is of great importance in saving lodged grain, is due to the fact that the nodes of cereals preserve the ability of renewing growth for a considerable time. As soon as the stem has been bent into a horizontal position, the lower side of the node starts growing, thus forming a curvature and raising the part of the stem situated above it.

If a seedling is placed in a horizontal position and is rotated

slowly on the klinostat, no curvatures will be formed, as by such rotation the unilateral effect of the force of gravity is removed. But if previous to setting the klinostat in motion, the seedling is left for several minutes in a horizontal position, not so long, however, as to prevent its beginning to bend, then after a certain time it will form a curvature on the klinostat. The former upper side of the stem will become concave, while the root will be convex. In other words, the shoot will bend in the same way as if it were left motionless in a horizontal position.

This experiment clearly proves, that in geotropism there is in operation a phenomenon of the same nature as in phototropism. Owing to the unilateral effect of the force of gravity, a condition of excitation is evidently produced in the plant, which is not equally distributed between its upper and lower sides. After a certain period of time, this leads to a definite reaction, the formation of a curvature, in spite of the fact that during this period the cause which produced excitation—the unilateral effect of gravitation—was removed by means of slow rotation. The least duration of stimulation necessary to produce a visible curvature is called the "presentation time." Usually it is very short, amounting in the most sensitive plants, like the stem of the sunflower, but to 2 to 3 min., while the time of reaction requires 45 to 60 min., and sometimes even several hours.

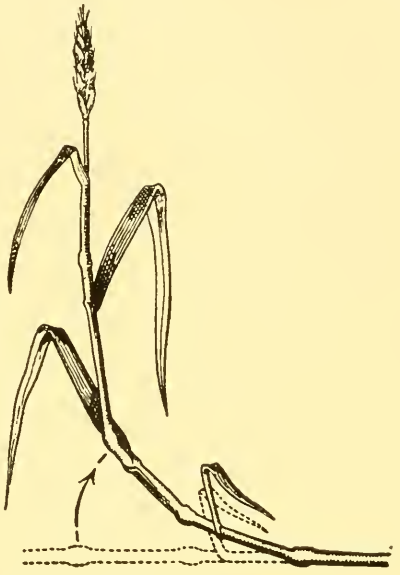


FIG. 133.—The rising of a cereal plant due to renewed growth and a curvature formed by the nodes (*after Nathansohn*).

The force of gravity is quite uniform at every point on the earth's surface. Therefore, it is impossible to reduce it to a certain dosage, as we may the intensity of light in studies of phototropic irritation. By changing the angle of deviation from the vertical line, however, it is possible to modify the intensity of geotropic

stimulation, which appears to be in proportion to the sine of the angle of deviation. This is known as the law of the sine. Therefore, the time of presentation seems to be least in a horizontal position and increases with the gradual approach to the vertical line. The product obtained by multiplying the time of presentation by the sine of the angle of deviation is a constant quantity.

By changing the angle of deviation, it is possible only to decrease the strength of the geotropic stimulus. In order to increase it, it is necessary to apply the effect of a different force, which also produces an acceleration of mass, namely, centrifugal force. If the shoots of plants are fixed in a vertical position on the circumference of a horizontally placed wheel which is rotated at considerable speed, it will be found that during further growth, the seedlings will deviate from their vertical position. Their stems will be directed towards the center, and the roots away from it. By altering the speed of rotation it is possible to change the angle of deviation from the vertical line. By this means it has been found that the direction of the axial organs will always correspond to the resultant between the force of gravity and centrifugal force.

In case of very rapid rotation, when the centrifugal force is greater than that of gravitation, the shoots will grow almost horizontally. And when the speed of rotation is such that the centrifugal force is equal to the force of gravity, then the shoots will grow at an angle of 45 deg.

By subjecting the shoots to the effect of centrifugal force during a short period and then transferring them to the klinostat, it is possible to obtain the same curvatures as are secured after a short exposure to the effect of gravity. It is also possible to determine a definite time of presentation for each intensity of centrifugal force. This time has been found to be in inverse proportion to the magnitude of the force used. In this case also one secures a full confirmation of the law of the quantity of stimulus which has been considered already in studying phototropism.

Geotropic stimuli are not equally perceived by all parts of an organ possessing geotropic sensitivity. As with phototropic stimuli, so here, the tip of the curving organ has been found to be the most sensitive part. The distribution of geotropic sensitivity may be most conveniently studied in the root. Charles Darwin (1880) showed that the tip of the root, or the last 1 to 2 mm., is the seat of sensitivity, and that roots with tips cut off grow hori-

zontally, having lost the capacity to perceive the direction of growth. In shoots of small grains, geotropic sensitivity is centered in the plumule, while in the majority of stems it is more or less equally distributed throughout the growing zone.

The question as to exactly how plants perceive the direction of gravity is of great interest, but can by no means be regarded as finally solved. The theory advanced by two scientists, Němec and Haberlandt, seems to have been popular for a long time. According to this theory, the perception of the direction of gravity

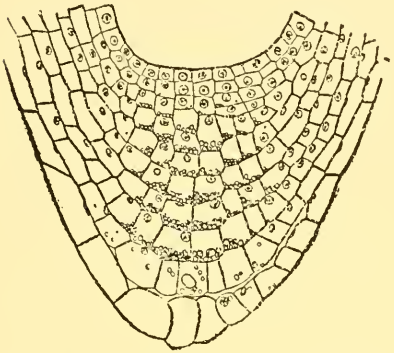


FIG. 134.—Statoliths in the root tip (*after Haberlandt*).

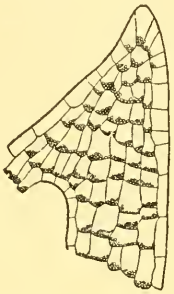


FIG. 135.—Statoliths in the cells of the plumule of millet (*after Haberlandt*).

is realized by means of mobile grains of starch found in certain cells. Having a relatively high specific gravity, the starch grains fall to that side of the cell which happens to be lowest and thus produce a pressure on the layer of protoplasm covering the wall of the cell. These mobile grains of starch are called "statoliths." Their greatest accumulation has been observed in the cells of the root cap (Fig. 134) and the tip of the plumule of millet (Fig. 135). In the stems of most plants, similar grains may be found in the cells of the so-called starch sheath or endoderm, which forms a cylindrical layer in the stem. This distribution of statoliths in certain parts of a plant is quite in accordance with the fact that in the shoots of cereals and in roots geotropic sensitivity is centered in the tips, while in stems it is spread throughout their length.

As a result of the discovery of new facts on the hormone nature of phototropism (Art. 94), the causes of geotropic curvature of

plants have been subjected recently to reconsideration. Experiments by Cholodny show that decapitated roots regain their lost geotropic sensitivity if the tips of roots or coleoptiles of corn are placed on the surfaces of the cut. Similarly, a section of the hypocotyl of lupine, which had lost its geotropic sensitivity (Art. 86), regains it if supplied with the growth-promoting substance from the coleoptile. It is evident, therefore, that the main factor influencing curvature and bending is the presence and distribution of this hormone. In a horizontally placed organ, or in case of one-sided illumination, the hormone may accumulate on one side, in this case the lower or ventral side, which, according to Browner (1927), becomes negatively charged. The cause of this deviation in flow of the substance, therefore, is probably the electrical polarization of the cells. The falling of the starch grains that carry a negative electrical charge may be considered as one of the factors of increasing polarization and not merely as the inevitable result of the force of gravity. Geotropical sensitivity of decapitated organs can be restored by fastening upon the surfaces of the cuts not only growing tips that manufacture the growth hormone but also pieces of paper or gelatin saturated with saliva, which may act as a substitute for the hormone (Art. 86).

The very important and interesting question as to why roots react differently to the force of gravity than do stems is explained by Cholodny on the basis of the different action of the hormone on roots as compared to stems. Under the action of the growth hormone, the rate of growth of stems is increased while that of roots is decreased. This is shown also by the fact that decapitated stems grow more slowly than uninjured ones, while decapitated roots grow faster than normal roots. Moreover, experiments have shown that the growth hormone which is secreted by the tip of the coleoptile of corn slows down the growth of positively geotropic organs and increases that of negatively geotropic organs. Although the growth hormone in horizontally placed stems and roots flows to the lower side, the result of its action is different. The accumulation of the hormone in stems increases the growth of the lower side and causes the organ to bend upward, while in the case of roots the growth of the lower side is decreased and the bending is downward. Thus the difference in geotropic reaction can be explained by a dissimilar reaction of the cells of roots and stems to the hormone that diffuses into them. Not all authors agree with the

above ideas in their explanation of phototropic sensitivity. Many think that under the influence of the unilateral action of gravity, special hormones are developed in the growing points, which may increase or decrease the rate of growth. All these questions are being studied actively at the present time, and it may be expected that in the near future the information on the nature of tropisms, as well as the general knowledge of growth phenomena, will be extended.

Geotropic as well as phototropic sensitivity is not to be considered as having constant properties. It may be influenced by both external and internal conditions. Thus, for instance, due to the effect of low temperature, negative geotropism of stems may change to transverse geotropism, and then the shoot will begin to grow in a horizontal position. This may be observed in spring in many plants, which cling to the earth during the early cold period and only later in the season begin to grow upwards. Similar changes of geotropic behavior may be seen also when plants are subjected to the effect of certain gases, ethylene and acetylene in particular. Plants appear to be extremely sensitive to these gases. According to Neljubov's investigations, 0.002 cc. of ethylene per liter of air is sufficient to cause the seedlings of peas or vetch to assume horizontal growth. In laboratories supplied with gas, therefore, it is impossible to obtain normal seedlings of such sensitive plants.

Similar changes in geotropic behavior are effected also by internal causes. Many rhizomes grow horizontally throughout the summer, but produce a vertical shoot the following spring. The peduncle of poppy exhibits positive geotropism before the period of flowering, and therefore its tip is directed downward. This is by no means caused by the weight of the bud, as it was formerly supposed, for in order to straighten the peduncle an amount of force far in excess of the weight of the bud has to be applied. However, towards the period of blooming its geotropic "behavior" is changed and the peduncle straightens out, due to the effect of the newly developed negative geotropism. The causes which produce these changes of geotropic behavior are not understood at present.

96. Chemo-, Hydro-, and Thermotropism.—Besides the force of gravity and light there are a number of other factors which may cause curvatures in the growing organs of plants, if they are sub-

jected to a unilateral exposure. These tropisms, however, have been studied very little. Moreover, their rôle in the life of a plant is comparatively small. Therefore, their discussion will be limited to only a short review.

Chemotropic curvatures are caused by an unequal distribution of certain substances in the environment of a plant. Chemotropism is of the greatest importance in the life of fungi and other saprophytic and parasitic plants. It assists them to direct their hyphae and haustoria to the source of nutritive substances. There are two kinds of chemotropisms, positive and negative. The former is caused chiefly by various nutritive substances, namely, sugar, peptone, asparagine, phosphates and ammonia compounds. Negative chemotropism manifests itself when acids and alkalies are applied. It may be produced also by certain salts, such as potassium nitrate and magnesium sulphate. A good method for the study of chemotropism in fungi is to sow their spores upon a perforated plate of mica, and placing it over a layer of gelatin which has been previously soaked in the substance that is being investigated. In the case of positive chemotropism, the fungus hyphae will penetrate through the perforations, but when negative chemotropism is exhibited they will draw away from the openings. The Japanese scientist, Miyoshi, has used leaves, previously injected with various solutions, for the same purpose. With this method, positive chemotropism caused the hyphae to penetrate through the stomata into the inner tissues of the leaf, a picture very similar to that observed in cases when the leaf is infected by a parasitic fungus.

The specific effect of various substances will depend on their concentration. Excessive concentrations may change positive chemotropism to negative. Fungal hyphae may exhibit a strikingly high sensitivity to some substances. Thus *Mucor* will show distinct curvatures when subjected to the effect of a sugar solution of as low a concentration as 0.01 per cent.

The growth of pollen tubes through the style to the ovule present a similar picture of chemotropism to that of fungal hyphae. They invariably find their way to the embryo sac, being guided, doubtless, by the substances secreted by the maturing egg cells. If a bit of an ovary be added to a drop of water with some pollen in it, the pollen tubes will be directed toward the ovarian tissues.

The chemotropism of roots has not been studied sufficiently.

The question presents considerable difficulties, as the same substance, diffusing in the environment of the roots, may produce a double effect on it. On the one hand, it may have a direct influence on the growth rate of that part of the root which comes in contact with the substance. And it may produce also a specific irritation in the highly sensitive root tip. The two effects may be antagonistic to each other. Thus, for instance, a toxic substance affecting the side of the root with which it comes in contact, produces a local inhibition of growth and, consequently a positive curvature. The same substance affecting the tip of the root may produce therein a condition of excitation which may be transmitted to the growing zone as a stimulus and may result in the formation of a curvature in the opposite direction. It has been possible to separate the two effects by means of a unique method of procedure suggested by Porodko. He placed the seedlings of various plants into a layer of gelatin through which the current of substances producing a chemotropic effect was directed. In some of the experiments, the tip of the root was left outside the gelatin; in others, the growing zone was outside. These experiments have led Porodko to conclude that non-electrolytes do not produce any chemotropic effect on roots, while the cations of electrolytes, as a rule, have a negative effect, and the anions a positive, the effect of salts being in this respect halfway between that of the cations and the anions.

Under natural conditions, the rôle of chemotropism in the direction of growth of roots is of little importance, at least as far as the effect of solutions in the soil is concerned. The distribution of air and water in soil appears to have a much greater effect on the growth of roots.

If a vessel containing the root system of a plant is hermetically closed leaving only one small opening, then the roots will grow in the direction of this opening to meet the current of oxygen. To this phenomenon has been given the name "aerotropism," it being but a special type of chemotropism. Humidity produces a still greater effect on the growth of roots. When it is not equally distributed in the soil, the roots may grow in the direction of the more humid portion. One may observe the bending of roots in the air towards more humid surfaces (Fig. 136). Such an experiment, however, will be successful only in an atmosphere which is almost saturated with water vapor, for otherwise the roots are apt to dry

out. But no hydrotropic curvatures will be secured in an atmosphere completely saturated with water vapor, as in this instance the whole space will be of equal humidity. Similar to geotropism, hydrotropic sensitivity is centered in the tip of the root.

An unequal distribution of temperature may also produce bending both of the roots and the aboveground organs, showing also both positive and negative thermotropism. When the temperature is below the optimum, plants will curve in the direction of the more heated side; when above the optimum, they will bend toward the colder side. Thermotropic curvatures may be secured with roots growing in moist sawdust.

The study of the reaction of plants to strong chemical and thermal stimulation is to a considerable extent complicated by the

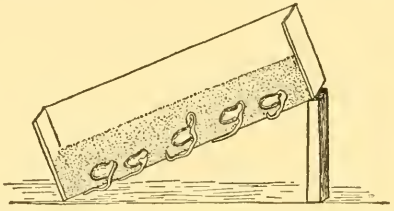


FIG. 136.—Hydrotropism of roots. Instead of growing straight downwards, the roots cling to the damp sawdust (*after Osterhout*).

phenomenon of traumatropism, which was discovered by Darwin. If the tip of a root is severely injured by means of a unilateral incision or cauterization with an acid, etc., the irritation produced is transmitted to the zone of elongation and produces a negative curvature. Traumatropic sensitivity permits the root to avoid obstacles, which may stand in its way. It is, therefore, of ecological importance. The aboveground organs, particularly of seedlings, also exhibit traumatropic bending, but these are chiefly of a positive nature.

The so-called autotropism holds an exceptional place among the tropisms. By this term is understood the tendency of a plant to grow in an upright position. This tends to counteract the curving effect of the other tropisms. Thus curvatures formed owing to a transient effect of some external stimulus are again straightened out, providing they have not been definitely fixed by growth. Autotropism also serves to straighten the small curvatures of a casual nature.

97. Movements of Twining and Climbing Plants.—The twining and climbing plants form a peculiar ecological group. In spite of their great length, they are characterized by very thin stems in which mechanical tissue is almost completely absent. Hence they are unable to sustain themselves in a vertical position and are in need of an external support. This is supplied by other plants, upon which they climb. According to the exact meaning of the word, the twining and climbing plants are not really parasites, since they develop no haustoria and feed quite independently. Nevertheless, they may do a great amount of harm to plants which they use as supports, by depriving them of light. This occurs the more easily, since plants of this group need but little substance for the building of their slender stems, and therefore develop very rapidly, easily outgrowing their supports.

The necessity to find a support upon which to lean has led the twining and climbing plants to develop a high degree of movement. They are, indeed, the most mobile plants among the representatives of our flora. According to the nature of the movements and the method of attachment to their support they may be divided into two different groups: the climbing plants, which attach themselves by means of special organs, the tendrils; and the twining plants, which coil around the support by means of their stems.

The most typical representatives of the climbing plants are the various cucurbits and legumes. Morphologically, their tendrils are either metamorphosed lateral shoots, or leaves or parts of leaves. These morphological distinctions are, however, of small physiological importance. In all instances the tendril consists of a thin lash, which is originally coiled as a spiral, but then extends and describes peculiar circular movements in the air (Fig. 137). If in the process of these movements the tendril meets with a firm support, it will clasp firmly around it and then, coiling up like a spring, will draw the shoot to the support. If they meet with no support, they wither.

Two kinds of movements are thus found in tendrils: the circular movement, while seeking a support, and the coiling movement around it. The first is autonomic. It is dependent on internal causes and is produced by an accelerated growth of the tendril on its various sides. Similar differences in the rate of development may be observed in all growing organs, producing a swaying of the growing tip, which is not possessed of any peculiar orientation.

In climbing plants, these movements are especially marked. The clasping of a support is a tropism, or the acceleration of growth on a definite side of the tendril, in this case of the external, more convex side, in response to an irritation. The stimulus is of the nature of contact or scraping, and is in many ways similar to irritation by touch in animals. The response to it on the part of the plant goes by the name of thigmotropism.

The thigmotropic sensitivity of tendrils is extremely high. A transient touch with the thinnest hair, weighing no more than a thousandth part of a milligram, is sufficient to produce an appre-

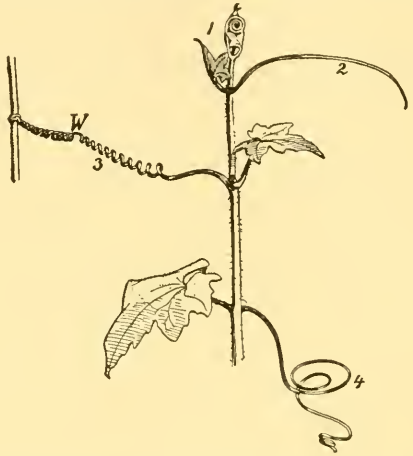


FIG. 137.—Tip of the stem of *Bryonia* with tendrils: (1) a tendril coiled into a spiral; (2) a tendril in a condition of highest sensitivity; (3) a tendril clasping the support and pulling the stem; (4) a tendril not attached to a support and therefore dying (*after Pfeffer*).

ciable bending of the tendril. In this instance the presentation time is also relatively short and the tendril continues curving after the removal of the object which caused the irritation. It is of interest to note, however, that in order to produce irritation a contact with a solid body is absolutely necessary. Drops or streams of water and even of quicksilver do not produce any thigmotropic curvatures.

If the stimulus is weak and of short duration, the curvature formed is again straightened out by the force of autotropism. If the support remains in place, new parts of the tendril come into contact with it in the process of clasping round the support and the

irritation is increased. Finally, the tendril may coil several times around the support, and thus become firmly attached to it. Then it rapidly begins to grow in diameter, at the same time forming a considerable amount of mechanical tissue which is never produced in a tendril that has not come in contact with a support. Thus, an irritation caused by contact not only produces a curvature, but the formation of new tissues as well. As soon as the clasping of a support has been completed, the rest of the tendril begins coiling into the shape of a corkscrew, becomes thicker, and draws the stem to the support.[1]

The capacity to perceive a stimulus is localized almost exclusively on the concave or the so-called ventral side of the tendril. A touch on the dorsal side will produce no curvature. Apparently, there are present special cells, provided with sensitive pores, which serve the purpose of perception. Such a pore consists of a small canal in the external membrane of the cell. This canal is filled with protoplasm. It is supposed that an external pressure is most readily perceived, where the membrane is thinnest. Similar cells, however, have not been found in all plants, consequently, they do not seem to be indispensable structures for thigmotropic sensitivity.

Twining plants do not have sensitive tendrils. They attach themselves to supports by means of quite a different mechanism. Not having any special organs of grasping, they twine around the support with their whole stem. The latter is characterized by very long internodes and a retarded development of leaves. The tip of the stem is therefore stretched into the form of a comparatively long lash. The upper part of the stem acquires a horizontal position and slowly rotates on the lower upright part, thus describing wide circles. These circular movements are of the nature of autonomic nutations, similar to the seeking movements of tendrils, but the grasping of the support by twining plants depends on other causes. On meeting with a vertical or inclined support, the stems of twining plants simply continue their rotating movement and thus finally find themselves twined around it. No thigmotropic irritation, with a subsequent unilateral growth and thickening, has been observed in twining plants.

The horizontal position of the tip of twining plants is due to

[1] The coils of the spiral are reversed at the center, which obviates the necessity of rotating either plant or support to make the spiral.

transverse geotropism, which changes in the more mature parts into negative geotropism. If a twining stem remains without a support, it stretches and straightens out the previously formed curvatures. But if it has clasped around a support, the coils of the spiral will cling closely to it, due to the same kind of stretching. The importance of geotropism in twining plants is indicated by the fact that they do not form spirals when placed on a klinostat. Moreover, they are able to twine only around a vertical or slightly inclined support, but never around a horizontal one, as the rotation of the tip is always in the horizontal plane.

The twining and climbing plants of the temperate zone are usually herbaceous annuals. They are of a comparatively small size, the only exception being the hop and the clematis. In tropical and subtropical countries may be found many woody plants of this type, the so-called lianas, which closely entangle trees, thus making the tropical jungles quite impassable.

98. Nyctinastic and Seismonastic Movements.—By tropisms are meant such growth movements as are produced by a unilateral effect of some external factor. They usually result in a definite orientation of an organ with respect to the stimulus, causing most frequently either a positive or a negative curvature. The term "nasties" is applied to such movements that are produced by stimuli, which have no definite direction, resulting in no particular orientation of the plant.[1] Such stimuli are, for instance, changes in temperature or illumination. A dorsiventral or bilaterally symmetrical structure of the curving organ is indispensable for movements of a nastic nature. Movements involving changes in turgor, otherwise called alternating movements, are here of far greater importance than in tropisms.

Nyctinastic movements caused by succession of night and day are most common. Many flowers open in the morning and close for the night; some leaves, particularly the compound leaves of papilionaceous plants, change their position during the night and day. These movements occur with considerable regularity, which led Linnæus to attempt to construct a "floral clock," by making a bed of plants in which the different flowers would open and close at various hours of the morning and evening. Movements of this nature are often said to be connected with the

[1] In nastic movements, the direction of the stimulus does not determine the direction of response.

"sleep of plants." This expression is inappropriate, as such nyctinastic movements have nothing in common with the sleep of animals, which is a peculiar condition of rest.

Changes in temperature or the intensity of light, or both, are the actuating causes of these movements. The first instance is concerned with thermonasty; the second, with photonasty. The opening of the tulip, or saffron, *Crocus sativus*, flowers, which may be easily observed when these plants are carried from a cold into a warm room, are typical instances of thermonasty. Under these conditions the flowers will open in 3 to 5 min. (Fig. 138). The

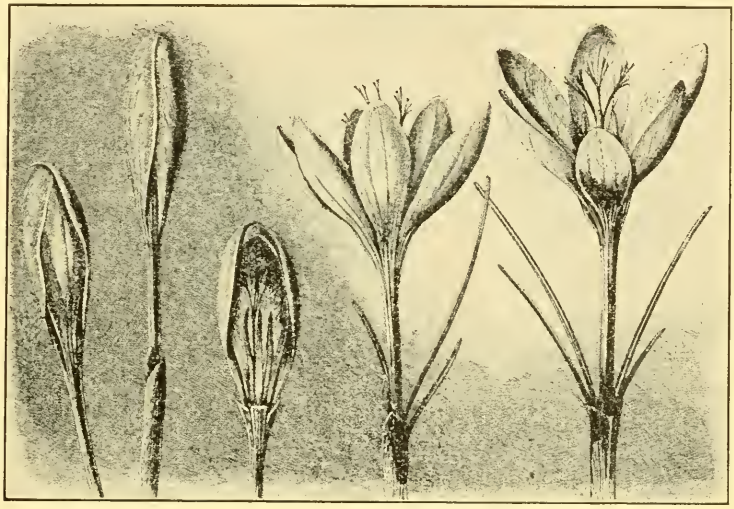


FIG. 138.—Thermonastic opening of saffron flowers (*after Kerner*).

mechanism of this movement lies in the fact that with an increase of temperature the growth of the inner surface, morphologically the upper surface, overtakes the growth of the lower surface of the petals, which then curve externally and the flower opens. When the plant is left for a considerable period in a warm temperature, however, then the growth of the lower side also will be accelerated, and the flower will again close.

The movements of petals of the water lily and of flowers of the dandelion are good examples of photonasty. Changes in the intensity of light make these plants close not only in the evenings, but in dull weather as well (Fig. 139). It may be produced also by

artificial shading, as for instance, by carrying the plant into a weakly illuminated room. Some plants, like *Nicotiana* and the four-o'clock, on the contrary, close their flowers when the light is increased and open under weaker illumination. They are fully expanded therefore in the evening and in cloudy weather.

Often the effect may be due both to temperature and illumination. The movements of the compound leaves of woodsorrel (*Oxalis*), clover, and other plants are thus produced. In some of these plants the leaflets droop during the night, in others they rise. These movements are produced by means of articulations of the leaves. They are of an alternating nature and are due to turgor changes in the upper and the lower halves of the nodes.

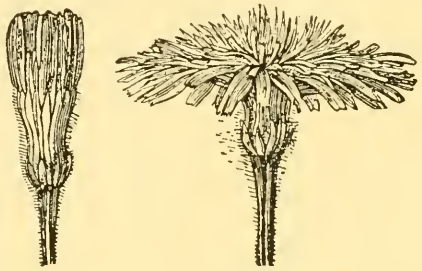


FIG. 139.—Photonastic opening of a dandelion flower head (*from Bonner Lehrbuch*).

Drooping movements are usually accompanied by an increase of turgor in the upper half of the node and a decrease in the lower half, while a lifting movement is produced by a reverse mechanism.

Nyctinastic movements of plants have an ecological rôle. In flowers, they facilitate pollination in favorable weather and protect the inner organs of blossoms during unfavorable conditions. Nyctinastic changes in position of leaves are thought to be of importance as a protection against chilling at night. But since they are most common in the tropics where cooling at night presents no danger to the plant, this explanation should be accepted with caution.

Of all the nastic movements, the greatest attention has been paid to those of seismonastic nature, which occur in response to a shock or a concussion experienced by a plant. The best-known illustration is supplied by the sensitive *Mimosa pudica*, which rapidly droops its leaves when touched, all of its leaflets folding in

pairs (Fig. 140). If the plant is severely shaken, then all its leaves will droop at once. A very strong irritation of a separate leaf, as by injuring through cauterization or by a stroke, will result in a rather rapid transmission of the irritation throughout the plant. As the wave of irritation spreads through the branch, the leaves will close one after the other. This wave may be transferred from

FIG. 140.—*Mimosa* branch. On the left, two open leaves. On the right, a leaf lowered and folded, due to irritation (*after Nathansohn*).

one branch to another and thus finally will involve the whole organism.

If the temperature is sufficiently high, above 25 to 30° C., then the leaves of *Mimosa* will fold and droop within a few seconds. Under these conditions the irritation received is also transmitted further with great rapidity, as fast as 15 mm. per second. Such an extremely rapid response is not characteristic of plants in general. Naturally, then, *Mimosa*, which was first brought to Europe from Brazil in the seventeenth century, very soon became widely known not only in scientific circles, but among the public as well. The sensitivity of this plant is very much akin to the irri-

tability of animals. It may be suppressed either by a lowering of temperature to below 15° C., or by various anaesthetics, especially the vapors of ether or chloroform.

What seismonastic sensitiveness really consists of has not yet been discovered. The only thing so far that has been established is that it is unequally distributed through the plant and that the most sensitive part is the lower surface of the pulvinus, the swollen base of the petiole. The mechanism of the movement consists of a change of turgor in the upper and lower halves of the pulvinus which supports the leaves of *Mimosa* (Fig. 141). An irritation suddenly alters the permeability of the protoplasm in the lower half of the pulvinus, *A*. The cell wall, which was formerly in a condition of tension, not being distended by the protoplasm, contracts. At the same time part of the cell sap is pressing out into the intercellular spaces. This results in a rapid decrease of turgor in the lower half of the pulvinus. As the upper half *B* preserves all of its tension, the petiole will bend downwards and the leaf droops. This extrusion of sap from the cells into the intercellular spaces is indicated by the lower half of the pulvinus becoming transparent. If a shock is produced in a leaf that has been cut off close to the stem and left to recover in a humid atmosphere, then it may be observed that the pulvinus curves and that a drop of liquid appears at its cut end. As the reaction passes, the liquid is absorbed by the leaf and the pulvinus again straightens out.

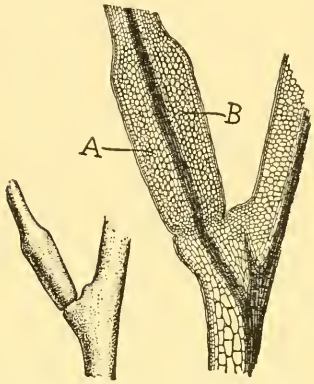


FIG. 141.—The pulvinus of a leaf peduncle of *Mimosa*. On the left, showing external view. On the right, *A*, section of the lower half of the pulvinus; *B*, section of upper half (*after Transeau*).

The transmission of stimulus in *Mimosa* occurs with such rapidity that it cannot be explained as being due to diffusion of a hormone, which after its formation in the irritated cells, must be moved through the parenchyma tissues of the stem. Moreover, it has been observed for some time that the excitation may be transmitted through dead sections of the stem, and that, even if the stem has been cut across and then joined together by a glass

tube filled with water, irritation nevertheless will be transmitted through this bridge of water. This has given rise to a theory that it is not excitation which is transmitted, but a hydrostatic pressure, similar to the transmission of a wave of compressed air in the tube of an air hammer. Recent investigations by Ricca, however, have somewhat contradicted this theory. They have confirmed the fact that irritation is transmitted through dead sections of tissues and through glass tubes. Still, Ricca believes that a hormone originates in the stimulated cells and that its transmission is not through the living cells, but through the vessels. These findings, however, require further confirmation.

Seismonastic sensitivity is present also in a few other plants though in a much lower degree. It is exhibited by the leaflets of woodsorrel and other representatives of the *Oxalidaceae* and by some of the leguminous plants, including the common locust. It is found in the leaves of certain insectivorous plants, particularly in *Dionaea*. The leaf blades of the latter consist of two halves, joined by a sort of hinge, which rapidly shuts when the peculiarly sensitive hairs distributed in the central portion of each half are touched (Fig. 95). It should be noted in passing that the seismonastic movements of insectivorous plants are almost the only instances of movements of this type. Their ecological importance, the catching of insects, is quite well known. The rôle of the various other movements, those of *Mimosa* included, are, however, not clear. At present one has to be satisfied with rather doubtful explanations, such as, for example, that the folding of the tender leaves of *Mimosa* caused by the first drops of rain protects them from being injured by a tropical shower, or that these movements scare caterpillars and other animals which feed on their foliage.

Seismonastic sensitivity is also shown by flowers of certain plants. In many flowers of the family *Compositae*, such as the cornflower, the stamenal filaments exhibit considerable contraction if touched. The mechanism of this contraction is similar to that found in *Mimosa*, *i.e.*, a rapid increase in the permeability of particular cells. A touch on the stamens of the barberry causes them to bend rapidly and press against the stigma. In certain plants (*Mimulus*, *Martynia*) similar movements of the fringed stigma may be observed. All these movements serve to facilitate a successful pollination of the plant.

CHAPTER XII

REPRODUCTION

99. The Normal Life Cycle of Higher Plants. Annual and Perennial Plants.—The life cycle of a higher plant, as well as of any organism, begins with the primary division of the fertilized egg cell and ends with the death of the individual. The early part of the life cycle is characterized by growth and the development of organs. This is succeeded by the stage of reproduction, which is followed by senescence and death.

The length of life of plants varies within broad limits. If only the higher plants are considered, there may be found ephemerals, like *Stellaria media*, which complete their development within a few weeks; very large trees, attaining an age of several hundred years, as oaks and linden; and those living several thousand years, as *Sequoia* in California. It should be emphasized here that there is a great difference between animals which have attained senility and plants in the same state. In an animal organism, almost all its tissues and organs are of the same age as the organism itself, while in a thousand-year-old tree all its active parts, the leaves, buds, tips of shoots, and roots, serve only for a short time and are then superseded by new ones. Thus, for instance, the leaves of a century-old oak tree are only a few months old. Only the innermost layers of the trunk of the tree, which usually are dead, may have originated in the distant past.

As a rule, trees and other perennial plants have the capacity of rejuvenating annually. They, therefore, have no definite length of life. Some of their parts die annually and are again restored. The cells of the embryonic regions of a thousand-year-old tree are as young as those of a year-old seedling. At any rate, it has not yet been possible to discover in many the indisputable symptoms of aging. It is, rather, the definite relationship between the vegetative growth of a plant and its reproduction, therefore, which is characteristic of its life cycle, and not so much the total duration of its life.

From this point of view, plants may be divided into two large groups—those fruiting only once and those fruiting repeatedly. To the first group belong primarily the so-called annuals which complete their life cycle within one vegetative period. For the sake of convenience, the cycle of development will be considered not from the moment of embryo formation, but from the time of germination of the seed, thus leaving the peculiar period of seed development out of consideration. The following are characteristic steps in the life of an annual: the seed germinates, the young plant rapidly develops its vegetative organs, then it proceeds to blossom, fruits are set and ripen, and finally all the organs of the plant begin to age rapidly and die. The plant then dries up. As the period of drying frequently coincides with the autumnal season, it may seem, therefore, that the death of such a plant is determined by external conditions. In reality, however, it is dependent on internal causes. Many early ripening annuals, for instance the various early varieties of crop plants, produce seeds and dry up long before the end of the growing season. This behavior is still more marked in the ephemerals.

Blossoming and fruiting do not always occur during the first year. Often it is accomplished in the second year of the life of a plant. This is true of all biennial plants, such as carrots and beets. They live through the winter in a state of rest, resuming growth only the following spring (Fig. 142).

Fruiting of these plants is also followed by death. There are some plants that develop for many years nothing but vegetative growth, attain a considerable size, and then begin to produce fruit heavily. In such cases, likewise, fruiting is followed by drying up of the plant. The agave, sometimes called the "century plant," belongs to this group. In Mexico, its native land, it will begin to blossom at the age of 8 or 10 years. Under less favorable conditions of development, as on the coast of the Mediterranean Sea or in greenhouses, a more protracted period (20 to 50 years) is required for its development previous to blossoming (Fig. 143). Some of the palm trees, among them the famous shade palm of Ceylon (*Corypha umbraculifera*) display the same characteristic behavior.

It has not been possible as yet to determine what inner processes cause the dying of plants subsequent to fruiting. But the fact that there exists a close causative connection between the two

has been proved by numerous experiments. The life of many plants may be considerably prolonged by removing their floral buds and thus preventing fruiting. By this method it is possible, for instance, to make an ordinary garden mignonette live 2 or 3

Fig. 142

Fig. 143

Fig. 142.—Stages in development of the wild carrot, a biennial plant. On the left the seedling; in center, plant at the end of first growing season, having formed a fleshy root. On the right, a mature and blooming specimen in the middle of the second year (*after Transeau*).

Fig. 143.—Century plant (*Agave*) showing rosette of fleshy leaves and flowering stalk (*after Transeau*).

years instead of one year (as has been done by Molisch). It will then acquire the form of a small shrub. On the other hand, when biennial plants, for some reason or other, produce seeds during the first year, the so-called shooting of beets and other root crops, then their vegetative organs will die promptly.

Other types of plants are characterized by repeated blooming. Here belong the great majority of perennials and woody plants. They blossom and fruit every year. Still that does not prevent their further vegetative growth. The duration of their life is practically unlimited. What inner physiological readjustments enable them to continue their vegetative growth after fruiting is at present unknown.

In general, there is a certain antagonism between vegetative growth and reproduction, which is especially marked in plants that bear fruits but once, and less manifest in plants that bear fruit repeatedly.[1] This antagonism is frequently attributed to the struggle for the nutritive substances which have accumulated in the plant. Analyses have shown that in the sunflower or the corn plant, for instance, almost all of the nutritive substances previously stored in the stems, the leaves, and even the roots are transferred to the ripening fruit. This phenomenon can be observed to a still greater degree in root crops. This evacuation of substances, however, cannot be the only cause of dying, for if the working capacity of the leaves and the roots were preserved, these losses might be compensated easily. The cause of this behavior should be sought in the quantitative changes of some hormones, which regulate growth and other life activities of the plant. Dying of plants may be regarded as a phenomenon analogous to senescence of an animal organism.

100. Effects of Environment on Time of Fruiting. Photoperiodism.—The exact causes which initiate reproduction are unknown. Several methods, however, are known by means of which reproduction either may be accelerated or delayed, or made more abundant. A detailed study of these methods is not only of great practical value for accelerating the time of a harvest, but also of great theoretical interest, as by this means it may be possible to approach the solution of the question as to the causes which determine the time of fruiting.

It has been noted for some time that the conditions of nutrition of a plant have a striking effect on both vegetative and reproductive development. Light seems to be of the greatest importance in this respect. If a plant is grown under conditions favorable in all

[1] Many plants with a continuous type of growth, like the tomato, some crucifers and legumes, though flowering repeatedly, show a distinct antagonism between vegetative growth and sexual reproduction.

respects excepting for an insufficiency of illumination, the blooming will be considerably delayed and sometimes does not occur at all. Klebs, for instance, grew in diffused light specimens of *Glechoma hederacea*, which remained in a vegetative condition for several years. But when the plants were placed in a stronger light they soon began to blossom. Similar experiments have been successful with other plants also.

This effect of light, however, is more of an indirect than that of direct nature. Plants that have a considerable storage reserve of organic substances, especially carbohydrates, will blossom even in darkness. The hyacinth and other bulbous plants are good examples of such a behavior. Similarly, it is possible to obtain flowers on separate branches of a plant, which are placed for this purpose in a dark chamber, providing their organic connection with other parts of the plant remain intact and the rest of the plant is exposed to strong light. On the basis of these facts Klebs was led to the idea that light hastens the period of blossoming chiefly by assisting the plant to accumulate carbohydrates and that the latter is the direct cause of the change from the stage of vegetative growth to that of reproduction.

This has been confirmed by experiments with plants grown under different kinds of colored glass, each representing a certain part of the spectrum. In red light, plants flower almost as readily as in white light, while blue light delays blossoming, despite the fact that the formative effect of light depends chiefly on the presence of blue rays. But analyses have shown that plants grown in red light are much richer in carbohydrates, both starch and sugars, than those grown in blue light.

Besides the carbohydrates, the amount of mineral substances and various nitrogen compounds also are of importance in determining the time of fruiting. It has been noted for a long time that nitrogenous fertilizers increase the vegetative growth of a plant at the expense of its reproductive functions. All the other soil nutrients seem to have a similar effect, with the exception of phosphoric acid, which usually increases fruiting. According to Klebs it is not the absolute amount of particular substances which determines the time of fruiting, but their relation to each other. A preponderance of carbohydrates over nitrogen and mineral compounds leads to flowering and fruiting; a reverse relationship, to vegetative development; hence, by combining certain fertilizers

and by other cultural methods, one may produce in the plant either an accumulation of carbohydrates or on the contrary an increase in nitrogenous compounds, thus making it possible to control to a certain extent the development and behavior of plants. The results obtained by Klebs have been confirmed lately by Kraus and Kraybill's experiments with fruiting of tomatoes. Moreover, many investigators have observed that an artificial increase of the carbon-dioxide content in greenhouses will facilitate fruiting, since in such case the carbohydrate content of the plant is considerably increased. A number of methods used by fruit growers, for the purpose either of accelerating the flowering or making fruit production more abundant, are also based on the relationship between carbohydrates and soil nutrients. Transplanting, the cutting of roots, or a temporary neglect of soil tillage are a few of such practices. Such procedures, however, have the disadvantage of curtailing the general development of a plant. Some methods serve to check the removal of assimilates from the stem or the individual branches. This may be accomplished by girdling of the bark or subjecting it to a pressure by means of a wire, or by partial twisting or breaking of the branches. Probably the most effective method of increasing fruitfulness is the use of the tree girdle, which is being widely applied in Germany (Fig. 144). This girdle consists of a thin strip of zinc, which is firmly fixed around the bark of the stem by means of wire, thus producing pressure and interfering with the downward flow of organic substances. The swelling formed above such a girdle is an evidence of this fact. A belt of this type does no excessive injury to the bark. It can be removed when not wanted any more. The general increase in fruitfulness due to grafting should be attributed, at least partially, to an interference with the downward flow of organic substances at the constricted places.

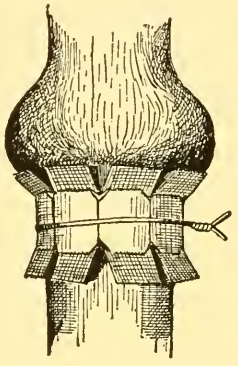


Fig. 144.—Tree girdle on a lilac branch. Note callus above the girdle (*after Molisch*).

The water relations in plants also have a certain effect on the determination of the time of fruiting. A dry air usually favors fruiting, while high humidity delays it or suppresses it entirely. A

dry soil, on the other hand, appears to be less favorable to fruiting, than a humid one. Consequently, the conditions that are most favorable to fruiting are those attained on well-irrigated land in an arid climate with a large number of bright days, as, for instance, in California, or Central Asia. Under such conditions fruit trees generally yield very large crops.

The effects of temperature on fruiting seems to be very complex. In general, a higher temperature is required for reproduction than for vegetative growth. It is possible to prevent plants from blossoming for a long period by growing them at a low temperature. But a temporary lowering of temperature to near freezing may serve as a stimulus for acceleration of blossoming and fruiting. This is most markedly evident in winter cereals. Under usual cultural conditions they will produce during the first year nothing but vegetative growth, even when sown in the spring, without a tendency to head formation before the beginning of the following summer. If the seeds are germinated at a temperature slightly above zero, however, winter cereals will blossom during the first year and, providing they have been sown sufficiently early, may even produce ripe grain. Lowering of the temperature during germination seems to serve as a stimulus, the effect of which continues even after the causal factor has been removed. Low temperatures produce a similar effect on biennial plants. Instances are known in which a cabbage or a beet, when nipped by frost, will start to fruit during the first year of its life. The mechanism of this effect is unknown.

Garner and Allard's recent investigations, which have been confirmed by a number of other investigators, show that very great importance in determining the time of blossoming should be attributed to the relationship existing between the duration of day and night. Some plants, as, for instance, many varieties of soybeans, kidney beans, the asters, and other plants, blossom when the days are short and the nights long. Consequently, under natural conditions these plants will produce flowers only in autumn when the days become shorter. By an artificial shortening of the day, as for instance, by placing the plants into a dark room during part of the day, or by covering them with opaque boxes, they may be made to bloom at the beginning of summer. Such plants have acquired the name of "short-day" plants (Fig. 145). There are, however, many plants, as for instance wheat, oats, and other

cereals, which will blossom sooner, the longer the day and the shorter the night. By illuminating them with electric light during a part of the night or all night long it is possible to make them bloom in midwinter. These are called "long-day" plants. Plants of this type have a shorter vegetative period in the northern latitudes, where the days in summer are very long and where the length of day overbalances the retarding effect of low temperature. These plants show the highest rate of development under conditions of continuous illumination.

The effect produced on plants by the relationship existing between the duration of night and day is known as "photoperiod-

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FIG. 145.—Tobacco plant, having grown the same length of time with (1) a "short" day, and (2) a "long" day (*redrawn after Garner and Allard*).

ism." It is of interest to note here that the nature of the plant's response to the length of day is to a considerable degree dependent on the origin of the plant. In the majority of cases, tropical plants are short-day plants, while those of the temperate zone belong to the group of plants requiring a long day. Hence, when transported to the tropics many of our plants will never blossom. The length of day is not only important in determining the time of flowering, it has also a marked effect on the development of vegetative organs. Thus, for instance, long-day plants will be conspicuously vegetative if the day is shortened to 12 hr. The plants will develop a much larger leaf surface and, in general, will

be more vigorous. Their functions of reproduction are thereby depressed. Short-day plants are influenced in the same way by the prolongation of the day to 16–18 hr. When cultivated in the north, where the summer days are very long, short-day plants, like millet, display great vegetative vigor, but flowering sets in late, and the crop has no time to mature. Here again may be observed the antagonism between vegetative and reproductive development, which has been mentioned before.

FIG. 146.—Development of *Rudbeckia* when exposed to a "long" day, *A*, and a "short" day, *B* (*after Garner and Allard*).

A very profound influence is exerted by the duration of the day on the development of tubers, bulbs, and other underground storage organs. A long day chiefly promotes the development of aerial organs, while a short day stimulates the formation of tubers and bulbs. Many tropical plants characterized in their native

country by an abundant tuber formation, like wild species of potatoes, *Oxalis*, etc., produce no tubers at all when grown in central Europe with its longer summer day. But by shortening the day artificially to 12 hr., numerous tubers are obtained. In the cultivated potato, tuber formation is also stimulated by a short day. For this reason it is especially intensive in autumn. In summer, with its long day, it is chiefly the vine which develops. As the production of a sufficient amount of starch requires a large leaf surface, the northern latitudes present especially favorable conditions for potato cultivation. During the northern summer, with its long day, the organs of assimilation develop vigorously, after which, with the considerably shorter day in autumn, a large amount of reserve substances is quickly accumulated in the tubers.

More recent investigations have shown that in photoperiodism the same after effect may be observed as under the influence of a lowered temperature. Thus, if the young shoots of millet, a short-day plant, are kept several days under the conditions of a long day and are afterwards exposed to a short day, it will be observed that the time of flowering is thereby considerably delayed, in comparison to plants grown continuously under short-day conditions, and that the vegetative organs have become strongly developed. The phenomenon of photoperiodic after effect makes us suppose that already in very early stages of development, the length of the day is able either to stimulate or to delay the formation of hormones specific for flowering, and that the subsequent course of development is to a considerable extent determined by the amount of hormones accumulated.

101. The Internal Factors of Fruiting.—External conditions do not directly produce or delay fruiting. They merely modify the internal condition of the plant, which, in turn, will produce either vegetative growth or reproductive organs. Even such a factor as the relationship between carbohydrates or nitrogen-free organic substances, and the amount of nitrogenous compounds and mineral salts, which is more of an internal than an external factor, cannot be regarded as an immediate cause of fruiting.

One of the most important inner causes determining the time of fruiting is the age of the plant. Many plants, particularly annuals, will begin to flower within 3 to 4 weeks after sowing, while some species of trees attain maturity only after several years. Thus, for instance, some oaks, elms, and chestnuts will not pro-

duce blossoms before the age of 40 to 60 years is attained, and some lindens, maples and larch trees will flower at the age of 20 to 30 years. What causes prevent them from fruiting at an earlier age is not yet clear. But it is possible to accelerate flowering considerably by various methods. When, for instance, plants are grown from cuttings taken from mature trees, they will start many years earlier. The same is true in cases of grafting, especially on dwarf stocks. Flowering and fruiting of trees growing in open spaces likewise occur several years earlier than in those growing in a dense forest. In this case, it is undoubtedly due to the effect of a more intensive illumination and a greater dryness of the air.

The fact that great changes occur in the different parts of a plant at the time of blossoming and that the nature of its further development is also somewhat altered, is of great interest and importance. Sachs (1883) observed that cuttings obtained from blooming specimens of the *Begonia* and other plants blossomed very soon, even before the plant had time to develop sufficiently; while similar cuttings taken from plants that were not in bloom produced for a long time nothing but vegetative growth. This phenomenon he explained by the fact that a certain "flower-forming substance" accumulates in the plant during the period of blooming. This substance then spreads throughout the organs of the plant. Consequently, when a leaf from such a plant is used for propagation purposes, flowering will occur very early.

Sachs's results have been repeatedly confirmed by other investigators. His hypothesis with regard to the special flower-forming substances has not been readily acknowledged. Since it has been established lately that many growth processes both in plants and animals are governed by specific chemical regulators, a number of investigators are falling back to the old belief advanced by Sachs and now seem to accept the idea that the essential cause, which leads to blooming, should be attributed to an accumulation of a specific hormone, all the other external and internal factors merely either contributing to or delaying this accumulation.

102. Physiological Processes Accompanying Fertilization and Embryo Development.—The process of fertilization and the subsequent development of the embryo have been studied in great detail, both morphologically and cytologically. The physiology of the process, on the other hand, has been very little investigated.

Because of its complexity it seems to be difficult to subject the process to a detailed physiological analysis. Only a brief mention will be made here of the most important achievements in this field. The consideration of fertilization will also be limited to the angiosperms, and the morphological and cytological side of the process will not be touched upon, since they are sufficiently described in textbooks of plant morphology and cytology.

Fertilization is preceded by the development of flowers which are metamorphosed shoots. Instead of green leaves, these shoots bear pistils, stamens, and a perianth. These organs are but little or not at all active in photosynthesis, but they have a very high respiratory rate. Therefore, in order that the formation and development of flowers, particularly in large numbers, may take place, a considerable amount of organic substances must accumulate in the plant. Consequently, in annual plants blossoming usually occurs after the middle of the vegetative period. In perennials, especially in a large number of trees, blossoming, on the contrary, takes place in early spring at the expense of organic reserves which have been accumulated and stored during the previous year.

Not being involved in the process of assimilation, the petals and sepals do not require much aëration and therefore have but few stomata. Hence, in spite of a thin cuticle, flowers always transpire much less than leaves. This explains why cut flowers wither so slowly.

As a rule, flowers grow and develop rapidly. Hence, as has been noted, even the opening and closing of flowers is of the nature of growth movements and not of alternating movements. Because of an almost continuous growth, flowers respire very intensively. The more massive flowers and inflorescences, such as the spadices of certain aroids, show a considerable rise in temperature as a result of a high respiratory rate.

Fertilization in higher plants is preceded by pollination, the transfer of pollen from the anther to the stigma. In the process of germination of pollen on the stigma, the external coat of the pollen grain (exine) is ruptured in definite places, while its inner coat (intine) is stretched into a long tube which descends through the style, thus reaching the ovule. The germination of pollen is in many ways similar to the germination of spores of fungi. Similar to fungal hyphae, pollen tubes show a pronounced chemotropism. The pollen of the majority of plants can germinate not only on the

stigma but also in artificial media, especially in sugar solutions at a concentration of 2 to 30 per cent. The pollen of a few plants can germinate in water, but in the majority of cases it will rupture and die when placed in this liquid. Most likely this is due to the very high osmotic pressure which exists inside the pollen grain.

The substances necessary for the development of the pollen tube, nitrogenous, as well as non-nitrogenous, are previously deposited in the pollen grain and the length of the pollen tube is evidently determined primarily by the amount of these reserves. A possible supply of nutritive substances to the pollen tube from without, *i.e.*, from the tissue of the style, is, however, not excluded. Besides, the growth of the pollen tube requires the presence of some specific substances which are supplied by the style. The pollen tubes of many plants will grow in the tissues of the style much better than in an artificial medium in which they will make but feeble growth. In many instances, the pollen will germinate only on the stigmas of the same species, and when it does germinate on those of another species, the growth of the pollen tube will be so slow that it never reaches the ovule. The pollen of many plants that require cross-fertilization produces very poor growth on the flowers of the same individual, while it may grow excellently on other specimens of the same species. It is supposed that certain inhibiting substances are formed by the plant which delay the growth of its own pollen, thus causing a physiological self-sterility in certain plants. It should be emphasized that this specific effect holds good for the pollen of all plants obtained by means of vegetative propagation from a single original individual. This fact is of great importance in horticulture and sometimes leads to low yields of fruit in an entire orchard.

The temperature conditions under which pollen germinates are also of considerable significance as regards successful pollination. There is a definite optimum temperature for the growth of pollen tubes, and the minimum is often quite high. Cold weather during blossoming time, therefore, has frequently an unfavorable effect on fruit setting. The harmful effect of heavy rains and fogs is due to the fact that pollen subjected to protracted wetting with water will burst before germination. Besides, sugar and other substances necessary for the germination of pollen may be washed off the stigma.

Since pollen is a resting spore, it will preserve for a certain period

its capacity of germinating. The period of viability depends on the kind of pollen, as well as on the conditions in which it is being preserved. The capacity of germination is preserved considerably longer in a dry and cool place. Under favorable conditions it is possible to preserve fertile pollen for several months.

The pollen of cereals, and of some other plants, represents another physiological type. The membranes of these pollen grains are readily permeable to water. In dry air such pollen rapidly desiccates and perishes. It must, therefore, be kept in a moist atmosphere. But even under these conditions its life is of short duration.

The question as to what factors help to prolong the life of pollen is also of great importance in practical work of cross-fertilizing varieties that blossom at different times of the year.

Having reached the ovule or, more exactly, the embryo sac, the pollen tube ruptures and the two generative nuclei contained in it effect fertilization. One of them unites with the nucleus of the egg cell, the other with the nuclei of the primary endosperm cell. When this has occurred, the process of the division of the fertilized cells begins. The embryo develops from the fertilized egg and the endosperm from what was the primary endosperm cell. If fertilization has not taken place, this division usually does not occur, except in cases of parthenogenesis, which will not be discussed here. Hence the assumption has been made that some inhibiting factor prevents further development in a mature egg cell, but is removed by fertilization.

What this inhibition factor is and how it is removed by fertilization are problems that have not been sufficiently studied. Experiments with animals and with free-swimming egg cells of certain algae have shown that artificial parthenogenesis, or the division of non-fertilized egg cells, may be produced by means of various chemical stimulants, especially certain mineral salts. This leads one to the conclusion that in higher plants the generative nucleus exerts a chemical stimulus which leads to further development of the egg cell.

Fertilization not only results in the development of the embryo and the endosperm, but also in a number of other changes, particularly in the ovule and other parts of the flower. The integuments of the ovule commence to grow and finally form the seed coat. The walls of the ovary develop and form the pericarp, while parts of the pedicel also frequently are involved in its formation.

At the same time the other organs of the flower, stamens, corolla, and calyx, die and either drop off or dry up.

Since most of the parts of the flower are not directly involved in the process of fertilization, most of these changes must be regarded as a peculiar effect on the part of the developing embryo or embryos, in cases of polycarpelate fruits. There is every reason to suppose that it is a matter of a chemical stimulation. The fertilized egg probably secretes certain hormones, which diffuse into the surrounding tissues and produce their growth.

It should be noted that in certain plants withering of some of the floral organs occurs even before fertilization has taken place or at the time the pollen tubes begin to penetrate into the tissue of the stigma. This phenomenon is evident particularly in the orchids, where the non-pollinated flowers remain fresh for a long time, but wither rapidly as soon as the pollen is transferred to the stigma. Fitting's experiments have shown that the same effect is produced when either dead pollen or that taken from a plant which is incapable of producing fertilization is used. This seems to indicate quite clearly that special substances diffuse from the pollen which cause the withering of certain parts of the flower.

In some cases, there may arise chemical stimuli which will cause the growth of the ovary even when fertilization has not taken place and the ovule does not develop. Under such conditions, the plant will bear seedless fruits, which are characteristic of some cultivated plants; the bananas, certain varieties of grapes, pears, apples, and other fruits. This phenomenon is known as "parthenocarpy."

103. The Rôle and Methods of Plant Propagation.—Fertilization gives rise to an entirely new organism formed by the union of the male and the female gametes. This organism, though very similar to its parents, possesses a somewhat different combination of hereditary units. This is particularly evident in cases of hybridization, when considerable differences exist between the paternal and maternal organisms. Besides sexual reproduction, there exists another common method of multiplication in the plant world. Various parts of the plant may separate from the parent body and grow into a new organism with all the organs peculiar to it. This method of multiplication is known as "vegetative propagation."

Vegetative propagation is merely a special type of growth of an organism. Certain parts lose their connection with other parts and

develop an independent existence. It may be observed in its simplest form in many perennial plants, whose rhizomes develop at one end and die off and decay at the other. This is commonly seen in the iris, the lily-of-the-valley, and many other plants. If a rhizome of this kind had formed branches, the parts which formerly joined the separate branches may eventually rot away and the branches then develop an independent existence.

Besides this indefinite form of propagation, one may observe in nature the formation of special organs serving the same purpose. Many plants produce long horizontal shoots, stolons or runners, either above or below the ground. These bear peculiar buds which root readily and attain a completely independent existence as soon as the connecting part of the stolon has been subjected to decay. A good illustration of such plants is the strawberry which usually produces several "runners." Bulbous plants often form lateral buds in the axils of their scalelike leaves, which can develop into independent bulbs. Small bulbs and tubers also may grow on various aerial parts of a plant, for instance in the leaf axils, as in *Ficaria* and certain lilies. In many varieties of onions they replace flowers in the inflorescence. The majority of these special organs of vegetative reproduction are characterized by an accumulation of various amounts of stored substances and by having a very pronounced rest period. In some viviparous plants, such as onion or meadow grass these bulbs germinate on the parent plant and, after dropping to the ground, continue their growth.

The conditions under which the organs of vegetative propagation are formed in higher plants have not been studied sufficiently. As a general rule, the most favorable conditions seem to be those that are most conducive to vegetative growth, namely, a high atmospheric humidity and weak illumination. Evidently, these conditions are exactly the opposite of those which promote sexual reproduction; hence, special organs of vegetative propagation are found chiefly in plants growing in damp, shady places, or in water. This type of propagation, therefore, may be regarded as a special supplement to or substitution for sexual reproduction.

Notwithstanding its long history, the question as to the exact rôle played in the life of a plant by each of these two methods of multiplication has not yet been finally solved. Likewise, it has not yet been determined whether plants may be propagated vegetatively an indefinite number of generations without the help

of the sexual process. There is a common belief that in case of asexual reproduction, the type or clone, will finally degenerate and that the sexual process must be employed in order to restore it. This idea, however, has not been definitely proven. Many instances of a complete loss of sexual reproduction are known in higher plants, such as yams, bananas or onions and yet such plants do not show any symptoms of degeneration. This is still more true with lower plants, fungi in particular, many of them having lost almost all traces of sexual reproduction.

Vegetative propagation, which is in fact merely a separation of certain parts of the parent plant, has the advantage of not requiring many of the frequently very complicated steps for securing a progeny (the transfer of pollen, etc.). This process, therefore, assures with greater certainty an uninterrupted existence of a species.

104. Propagation by Cuttings and Layers. Regeneration and Polarity.—Owing to a high degree of physiological independence which is characteristic of almost all parts of a plant and even of its separate cells, the latter have to a considerable extent the capacity for restoring all the lacking organs and of producing a completely new individual. This knowledge has been extensively utilized in agriculture, but particularly in horticulture. Since ancient times, a large number of methods for the artificial propagation of plants have been in practical use. By artificial propagation is meant such methods which do not require the use of any special organs of sexual or asexual reproduction formed by the plant itself, but which utilize any convenient and suitable vegetative part.

The most popular method of artificial propagation is by the use of cuttings and layers. By a cutting is meant any part of either the stem, the root, or even the leaf of a plant, which, having been separated from the plant and placed in favorable conditions for its development, is capable of growing into a new organism. If such a vegetative part is left organically connected with its maternal tissues, though only during the first stages of rooting and growth, then it is called a layer. Both of these methods are very popular among fruit growers. Layering is used in cases when cuttings do not root readily, as, for instance, in the case of grapes, hazelnuts, mulberries, gooseberries, etc. In order to obtain a layer, a branch is usually bent down and covered with soil for some

length, the tip being turned upward and left uncovered. The covered section in time will produce adventitious roots after which the branch may be cut off and the layer separated from the maternal plant (Fig. 147). Sometimes rooting does not occur under such procedure. Then it is necessary either to make incisions on the part of the branch that is covered with earth, or to girdle it. A callus is usually formed above the incision or girdle, from which roots will arise.

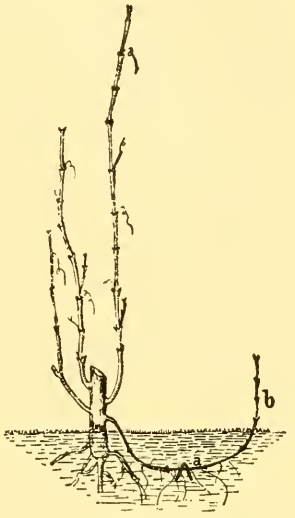

Fig. 147.—Showing a method of obtaining a layer (*b*) from a grapevine (*after Molisch*).

Cuttings usually consist of a section of the stem, either with or without leaves. Before being used their lower ends are dug into the earth to a certain depth. The soil used for this purpose must be very loose, preferably sand, and the cuttings have to be placed in an inclined position, so that their lower end should not be too deep in the soil. This is necessary, owing to the fact that the healing of the wound caused by the cutting and the subsequent formation of roots leads to an intensive respiration, therefore, a liberal access of air is indispensable. A high humidity is another necessary factor for successful rooting. This prevents the cuttings from drying before they have had time to develop their own root system. This is particularly essential for cuttings that bear leaves. Hence, previous to rooting, cuttings are often kept in greenhouses or in special boxes with glass lids, or they are placed under glass frames. Some cuttings, as those of willow, root very readily within a few days, while others, such as conifers, or camelias, require several weeks or even months.

Cuttings also may be obtained from the roots of certain plants. These are dug completely into the earth, in a somewhat inclined position. Adventitious buds will then be formed on their upper section, which will produce shoots, while the roots will be formed from the lower section.

A leaf also may be used as a cutting. It is planted with its peduncle into moist soil. In most instances this will result in

the formation of roots on the surface of the cut, just as in the case of stem cuttings. However, because of the absence of buds, no aërial organs are formed and such a leaf is incapable of developing into a new plant (Fig. 148). It only increases in size somewhat, and what is of particular interest will live much longer, even for several years, while on the tree it might have lived at the most one or two years. There are a few plants the leaves of which are able to form adventitious buds when they are separated from the mother plant. The best-known instance is the leaves of some of the *Begonias*. If one of their leaves is placed on moist sand and

FIG. 148. — Rooted leaf of *Aucuba japonica* (*after Molisch*).

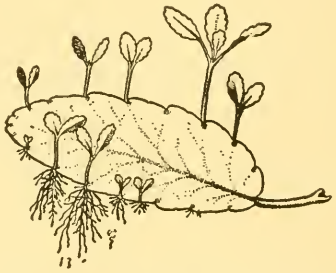

FIG. 149.—The formation of shoots on a *Bryophyllum* leaf (*after Transeau*).

the larger veins are cut through, buds will appear on the points of incision, which will later develop into new plants. This is a common gardener's method of propagating *Begonias* by means of leaves. Some varieties of *Cardamine*, *Bryophyllum* (Fig. 149), tomato, the hyacinths, and other plants have also the capacity for producing buds from leaf cuttings.

Cuttings which are separated from the plant even when very small in size may restore all the other parts of a plant. This is called "regeneration." Regenerational activity begins with the formation of callus on the surface of the cut tissues. This results from an increased division of cells adjoining the surface of the

wound. Even cells that had completed their growth long before the injury was performed may divide again under such conditions. Especially energetic is the formation of the callus in the immediate vicinity of the phloem. This has given rise to the supposition that the phloem carries special hormones which stimulate cell division. In the case of injuries, the hormones enter the cells adjoining the phloem, inducing an increase of growth and division.

The callus consists primarily of undifferentiated parenchyma cells in which the conducting elements, the cambium and regions of growth, are differentiated. The latter produce the adventitious roots. Numerous roots are developed also from the cambium of the cutting, but new shoots are formed with great difficulty from the callus. In order that propagation by cutting may be successful, it is important, therefore, that there should be on the cutting at least one bud capable of developing into a new shoot. In this connection, it should be noted that the so-called dormant buds, that normally would not have developed for many years, if at all, will produce shoots on a cutting.

If a small section is cut from the middle of a branch having two injured surfaces, one at the top and the other at the bottom of the cutting, the callus at the lower end will develop much faster and more extensively than that at the upper end. This, however, cannot be attributed to a downward movement of organic substances, as might appear probable at first sight, for if such a cutting is placed upside down, so that its morphological lower end will be at the top, then the callus will be still more pronounced at this end, though the force of gravity will act under these circumstances in the opposite direction (Fig. 150). When sections of axial organs react differently at their two morphologically opposite ends, the phenomenon is known as "polarity."

Polarity manifests itself clearly in the distribution of roots and the development of buds of a cutting. If a cutting is suspended in normal position in a humid atmosphere, the roots will be formed largely at its lower end, and the shoots will be produced from buds situated at its upper end; but when the shoot is suspended in an inverted position, the roots will nevertheless arise from the morphologically lower end, though it will now be at the top, while the shoots will develop at the morphologically upper end, now at bottom. If the center of the cutting is girdled, the effect will be the same as that of the division of the cutting into two separate

parts. This leads to the conclusion that polarity is most likely caused by the accumulation of some substances which move in the bark in a definite direction and which stimulate at the point of their concentration the development of both the callus and the roots. Sachs has named these "root-forming substances," by analogy to the flower-forming substances, which have been discussed (Art. 101). Some recent investigators have considered these to be hormones. According to Loeb, the root-forming hormones originate in the leaves, whence they descend through the stem, while substances which delay the growth of roots ascend through the stem. As long as a branch is attached to the tree, no formation of roots takes place; but, if the vessels through which the downflow of these hormones proceeds, are intersected, then the latter will accumulate at the surface of the cut and cause the formation of roots. It has not been possible, however, to isolate these hormones and produce with their aid the formation of roots.

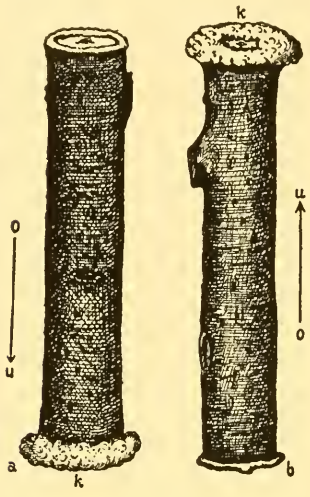

FIG. 150. — Poplar cuttings suspended in humid atmosphere. On the left, in normal position. On the right, in a reversed position, showing polarity in callus formation (*after Molisch*).

105. Budding and Grafting. Chimeras.—If a cutting of a plant is not allowed to root, but is set upon another plant, with which it is capable of uniting by growth, it will begin to develop and eventually produce a new organism. But the cutting under such conditions will not form its own root system. This operation is known as "grafting." The transplanted cutting is called the scion, and the plant with which it is united, the stock. Grafting is a common method of propagation in horticulture, as the properties of improved varieties are not inherited through reproduction by seeds, while the cuttings of many fruit trees do not root readily. Seedlings obtained from the seeds of wild apples or pears commonly serve as stocks for this purpose. They are usually cut off at the crown and a small cutting of an improved variety is then grafted upon the remaining stump.

If the stock and the scion are of the same diameter, the oblique surface of the one is placed over a similar surface of the other with the purpose of joining them by growth. This method is called "whip grafting." If the scion is much thinner than the stock, then cleft grafting is resorted to. The stock is split in two and the scion, cut to a sharp wedge, is inserted into the cut. To avoid a drying out of the junction it is sealed with grafting wax and sometimes firmly bandaged. Instead of a whole cutting, very often only a bud with a small section of adjacent bark and wood is used for propagation purposes. This shield-like cutting is inserted

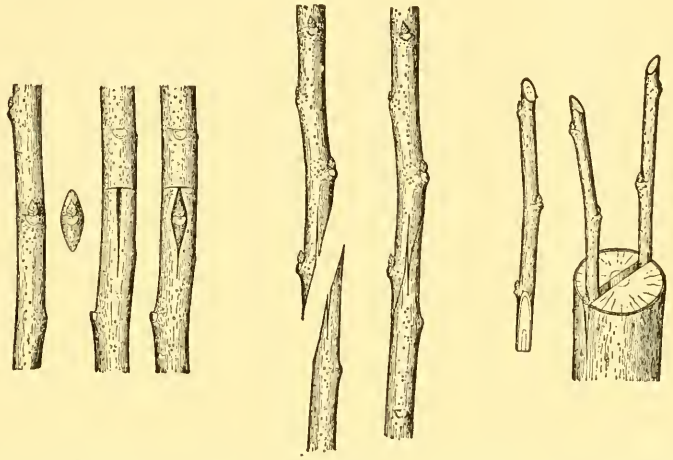

FIG. 151.—Methods of budding and grafting. Left, budding; center, whip grafting; right, cleft grafting (*after Brown.*)

into a slit made in the bark of the stock. This method is known as "budding" (Fig. 151). Various other methods of grafting and budding are known, but these will not be discussed here.

In all the methods of this type of propagation it is most important to ensure the closest contact between the cambium region and tissues of the same age of the stock and the scion, as only such tissues may be joined together by growth. The callus plays the part of an intermediary agent in the process of subsequent differentiation of vessels and sieve tubes, which serve to connect the corresponding elements of both plants. In grafting, the contact of the phloem elements is of especial importance. As has been seen, it is these elements which are the centers where the callus

formation begins and where it is most vigorous. Very often a thickening of the stem, which is caused by an excessive development of the callus, is formed at the place of junction of the stock and scion. The joining of the two parts will be the easier, the nearer they are related to each other. It is easy to graft different varieties of the same species, but it is also possible to unite two species of the same genus and even of different genera of the same family.

Various methods of grafting and budding have been used in horticultural practice since very ancient times. But only recently the subject has been put to a scientific analysis, chiefly through the investigations by Vöchting and Winkler. The most important question seems to be the reciprocal effect of the stock and the scion, the two together representing a united organism. The roots of the stock supply the leaves of the scion with mineral nutrients, while the leaves, in their turn, send to the roots organic compounds. Still, despite this very close relationship, the two parts of a grafted plant are independent of each other, each preserving its own peculiarities, as well as its specific storage products. Thus, for instance, in the sunflower, carbohydrates are stored in the form of starch, while in the artichoke (*Helianthus tuberosus*) it is in the form of inulin. If a sunflower is grafted upon the artichoke, tubers of the usual type are formed on the roots of the latter and these are filled with inulin, though the material for its formation is supplied by the sunflower. Sometimes, however, specific qualities are transmitted from one part of the graft to the other. Thus, for instance, in grafting tobacco upon the potato, the specific alkaloids of the tobacco are transported to the potato stock.

Still, in grafting, a quantitative effect is more common than is a qualitative one. Thus, in grafting an apple tree upon the Paradise stock or a pear upon the quince, the time of fruiting is hastened, which often is of great advantage. The duration of life, however, is shortened. An apple tree upon Paradise stock dies at the age of 20 to 25 years, while normally it may live for 200 years. Vöchting's experiments with beets are of exceptional interest in this connection. He grafted the adventitious buds, developing at the base of the inflorescence, into one- and two-year-old roots. In the first case they produced vegetative shoots, in the latter, flower-bearing shoots. If blossoming is induced by specific hormones, then this experiment must be interpreted as a diffusion of these hormones from the stock into the scion.

While a reciprocal effect of the stock and the scion undoubtedly exists, it must be, on the whole, insignificant. Thus, a score or two of choice varieties of apples may be grafted into the crown of a wild apple tree and each of them will preserve all its specific properties and will produce fruit of a quality characteristic of the specific variety. A preservation of the characteristics of a variety is only possible because the reciprocal effects between the stock and scion are very insignificant.

Chimeras, which at one time were called "graft hybrids," supply the most evident proof of the fact that each component of a graft preserves its own specific properties in spite of their close connection. They have been studied in detail by Winkler. By chimeras are meant plants which are sometimes obtained as a result of grafting one plant upon another and securing a combination of tissues of both. At first it was supposed that in these cases a fusion of cells had taken place, similar to that occurring in fertilization, hence the name "graft hybrids." Winkler's investigations, however, have shown that it is not so. He determined with accuracy both the nature of chimeras and the method of obtaining them. Chimeras are easily obtained from various species of *Solanum*, especially *Solanum nigrum* and *Solanum lycopersicum*. The latter may be joined to the former by cleft grafting. A stump of the nightshade plant, *Solanum nigrum*, is split and into it is inserted a wedge-shaped tomato scion. They are then left to unite. When they have grown together, the scion is cut near the union. This results in the formation of adventitious buds on the cut surface. Those formed from the surface of the stump will develop shoots of *Solanum nigrum*, while those arising from the wedge will produce tomato shoots. Sometimes buds are formed at the point of junction of the stock and scion, however, and then they will develop into shoots of a combined nature. These will be chimeras, in which one half of the tissues will belong to one plant, the other half to the other. And if, for instance, the boundary line passes across a leaf, the latter will be of an asymmetrical form (Fig. 152, 2). Such chimeras are called "anticlinal."

Winkler has obtained chimeras of a still more peculiar nature. Sometimes the buds were so formed that the outward layers of the growing points belonged to one plant and the inner tissues to the other. As a result, all the organs developing from these buds consisted of two kinds of tissues, the external tissues of one kind

and the internal of another. According to their external appearance such chimeras, called "periclinal," show an intermediate character between the two species (Fig. 152, 3), but cytological investigations have shown that the tissues of each component preserve the number of chromosomes characteristic of it, as well as other peculiarities; hence, even in this close union, the reciprocal

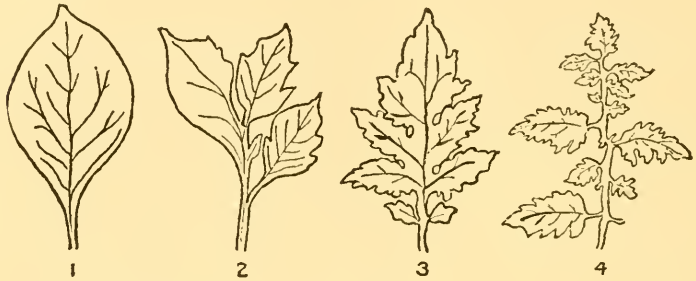

FIG. 152.—Black nightshade and tomato chimeras. 1, Leaf of a normal black nightshade; 2, leaf of an anticlinal chimera, 3, leaf of a periclinal chimera; 4, leaf of the tomato (*redrawn from Winkler*).

effect of the two species that are closely joined by growth is not very great.

Other types of chimeras have been obtained, and it has been established that some varieties which have been reproduced vegetatively for a long time, for instance *Cytisus Adami*, many varieties of *Perlargonium*, some clones of potato, etc., are chimeras with their inner tissues belonging to one plant, and the exterior to another. While the fruits of such chimeras are usually of a mixed nature, the seeds always belong to the plant which forms the inner tissues. Chimeras may be preserved only by means of vegetative propagation.

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